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MOISTURE ABSORPTIVITY OF THE POLY(LACTIDE-*CO*-GLYCOLIDE), AND COMPARISON OF DEHUMIDIFYING GASES

Master of Science thesis

Examiner: Professor Minna Kellomäki
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ABSTRACT

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The potential of the biodegradable polymers have been realized in the orthopedic and cosmetic applications world, due to their remarkable ability to degrade *in vivo*, why there is no need for second surgery. Poly(lactide-*co*-glycolide) (PLGA) is one of the most studied and used biodegradable polymer in orthopedic field, and the aim of this study was to examine the water absorption rate of PLGA (according to ISO62:2008), and comparing different dehumidifying gases for the drying processes.

The average absorption rate of the material was calculated to be for the process phase 2 samples 2.18 m-% and for the process phase 1 samples 0.51 m-% during 24 hours, in 100 % RH and at 23℃. In 50 % RH conditions the process phase 2 samples absorbed 0.74 m-% and for the process phase 1 samples 0.17 m-% during 24 hours. Since the production has under 50 % RH conditions, there should not be occurring any major degradation in these temperature values.

According to the directional tests that had done, compressed dry air (CDA) could be potential alternative for the nitrogen gas in drying and preservating processes, since its capability to preserve moisture content of the samples was relatively similar to nitrogen. However, some advantages were noticed compared to nitrogen, such as, more efficient water removal capability of the CDA, due to reactivity of oxygen with the water molecules.

TIIVISTELMÄ

Pauliina Ala-Vannesluoma: Poly(laktidi-*ko*-glykolidi):n kosteuden absorptiokyky ja kuivaavien kaasujen vertailu
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Biohajoavien polymeerien potentiaali ortopediassa sekä kosmeettisissa sovelluksissa on huomattu niiden *in vivo* -ympäristössä tapahtuvan hajoamisen takia, minkä vuoksi vältytään useammilta kirurgisilta operaatioilta. Poly(laktidi-ko-glykolidi) (PLGA) on yksi tutkituimmista ja käytetyimmistä biohajoavista polymeereistä ortopedian alalla. Tämän tutkimuksen tavoitteena oli selvittää veden imeytymisen nopeutta PLGA:an ISO 62: 2008 standardin mukaan ja verrata vaihtoehtoisia kuivaavia kaasuja.

Kosteuden imeytymisen materiaaliin laskettiin olevan keskimäärin 2. prosessointivaiheen näytteille 2.18 m-% ja 1. prosessointivaiheen näytteille 0.51 m-% 24 tunnin aikana, 100 % suhteellisessa kosteudessa ja 23 ℃:ssa. 50 % suhteellisen kosteuden olosuhteissa 2. prosessointivaiheen näytteet imivät 0.74 m-% ja 1. prosessointivaiheen näytteet 0.17 m-% 24 tunnin aikana. Koska tuotanto pysyy alle 50 % suhteellisessa kosteudessa, suurta materiaalin hajoamista ei pitäisi esiintyä näissä lämpötilaoloissa.

Tässä työssä esitettyjen tulosten mukaan paineilma voisi olla mahdollinen vaihtoehto typpikaasulle, sillä sen kyky ylläpitää näytteiden kosteuspitoisuus on lähes samanlainen kuin typellä. Muutamia paineilman käytön hyötyjäkin huomattiin, kuten sen tehokaampi veden poisto verrattuna typpeen, mikä selittyy hapen ja veden välisen reagoinnin avulla.

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ABBREVIATIONS AND SYMBOLS

PLGA	Poly(lactide-*co*-glycolide)
CDA	Compressed dry air
MRI	Magnetic resonance imaging
PLA	Polylactic acid
PGA	Polyglycolic acid
FDA	Food and drug administration
ASTM	The American Society for Testing of Materials
ISO	International Standards Organization
T_g	Glass transition temperature
T_m	Melting temperature
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
PP	Polypropylene
J	Flow of the water through wood
∇	Gradient operator
D_{e}	Diffusion coefficient
RH	Relative humidity
w_1	Weight fraction of two component
w_2	Weight fraction of two component
T_{g1}	T_g of the heat component
T_{g2}	T_g of the heat component
χ	Parameter related to the strength of the interaction between the two polymers

CH_3OH	Methanol
SO_4^{2-}	Sulfate ion
RN	Base
$[RNH]SO_3CH_3$	Alkyl sulfite intermediate
I_2	Iodide
H_2O	Water
$[RNH]SO_4CH_3$	Alkyl sulfite
µg	Microgram
mg	Milligram
RSD	Reproducibility standard deviation
AK	Switch-on criteria
IV	Inherent viscosity
DSC	Differential scanning calorimetry
m_{wet}	Wet mass
m_{dry}	Dry mass
N_2	Nitrogen (gas)
O_2	Oxygen (gas)
CMS	Carbon molecular sieve

1 INTRODUCTION

Over the decades orthopedists have used metal as a material for medical devices in different orthopedic applications. Metals have several advantages: they are easy to handle, tough, and most importantly they have produced good clinical results. However, they have some drawbacks as well. For example, metals may limit the consecutive use of magnetic resonance imaging (MRI) and X-ray as a post-operative assessment tool and may start to corrode and generate wear-debris. These consequences usually requires a second operation. [1]

Biodegradable polymer has been introduced for the orthopedic and cosmetic applications, since biodegradable polymers degrade *in vivo*, which means that there is no need for second surgery and in that way there is an opportunity to save both money and time. Biodegradable polymers have already been used for example for suture, vascular clips, and bone fixation implants, and they have established their position as implantable medical devices. [2]

Especially PLGA is broadly used material for biodegradable implants. PLGA is a synthetic copolymer from poly(lactic acid) (PLA) and poly(glycolic acid) (PGA). Biodegradable PLGA has been broadly used in plates, sutures, and screws for repair of bone fractures and drug delivery systems [3]. PLGA degrades via hydrolysis *in vivo* into relatively harmless products such as lactate and glycolate [4].

Due to the hydrolytic degradation of PLGA, typical parameters including the glass transition temperature and molecular weight change when PLGA was exposed to moisture. Moisture content is the main parameter for the hydrolysis, since water molecules are the ones that initiate the whole degradation process. The rate of moisture absorption depends on the material type and the prevalent conditions such as temperature, humidity and contact time. Moisture absorption can affect in different material properties, such as dimensional stability and mechanical properties. [5]

The aim of this study was to examine the water absorption rate of the PLGA. After the absorption rate was determined, compressed dry air (CDA) was investigated and compared to nitrogen gas as a dehumidifying gas. This work is only one public part of the whole Master of science thesis.

2 LITERATURE REVIEW

2.1 Biodegradable medical device

A medical device is defined by Food and drug administration (FDA) "an instrument, apparatus, implement, machine, contrivance, implant, in vitro reagent, or other similar or related article, including a component part, or accessory which is:

- recognized in the official National Formulary, or the United States Pharmacopoeia, or any supplement to them,
- intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease, in man or other animals, or
- intended to affect the structure or any function of the body of man or other animals, and which does not achieve any of its primary intended purposes through chemical action within or on the body of man or other animals and which is not dependent upon being metabolized for the achievement of any of its primary intended purposes." [6]

The American Society for Testing of Materials (ASTM) and the International Standards Organization (ISO) define degradable polymer as a material, which undergo a remarkable change in their chemical structure under specific environmental conditions, which cause a loss of mechanical and physical properties. In addition, biodegradable materials are degraded *in vivo*, enzymatically, via hydrolysis or both, producing biocompatible, non-toxic by-products which are excreted via the normal metabolic pathways. [7] Main feature for biomaterial and its by-products is that they must be biocompatible. Biocompatibility is ability of the material to perform appropriate host response in a certain application. [8]

In European Union, the core of the legal framework of the medical devices consists of three directives: Directive 90/385/EEC [9] concerning active implantable medical devices, Directive 93/42/EEC [10] concerning medical devices, and Directive 98/79/EC [11] concerning *in vitro* -diagnostic medical devices. The latest versions are revealed in directive 2007/47/EY [12].

2.1.1 Polylactide and lactide copolymers

Polylactic acid or polylactide is a biodegradable thermoplastic aliphatic polyester. Its molecular formula is $(C_3H_4O_2)_n$, and it consists of alternating hydroxyl and carboxyl groups in the polymer chain. It exists as two optical isomers, D and L. These differ from each other by their properties. [13] The chemical structure of PLA is illustrated in Figure 1.

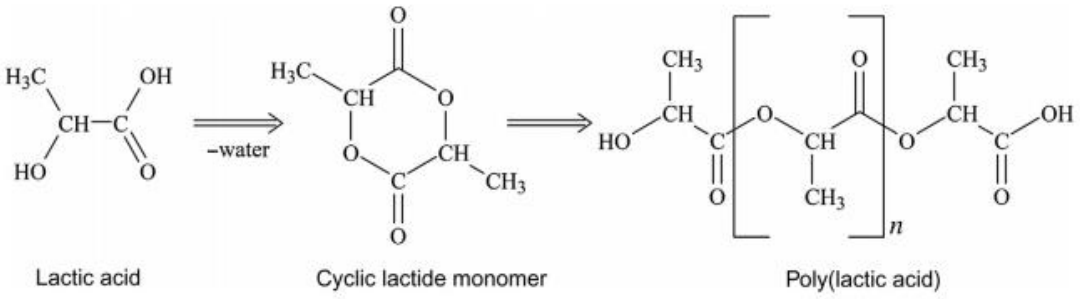

Figure 1. *Polymerization of PLA. [14]*

PLA has several interesting properties that makes it popular for many applications. Especially its mechanical strength, biocompatibility and degradability are giving many opportunities to use PLA in different products in the medical field. [15] Common degradation time for pure PLA is more than 12 months, depending on its configuration.

The properties of PLA depends heavily on the relation of the isomers (D/L) of the component, processing temperature, annealing time and molecular weight. Some of the key physical and chemical properties are shown in Table 1.

Table 1. *Physical and chemical properties of PLA. [16]*

Properties	PDLA	PLLA	PDLLA
Solubility	All are soluble in benzene, chloroform, acetonitrile, tetrahydrofuran (THF), dioxane etc., but insoluble in ethanol, methanol, and aliphatic hydrocarbons		
Crystalline structure	Crystalline	Hemicrystalline	Amorphous
Melting temperature (T_m)/ °C	~180	~180	Variable
Glass transition temperature (T_g)/ °C	50-60	55-60	Variable
Decomposition temperature/°C	~200	~200	185-200
Elongation at break/ (%)	20-30	20-30	Variable
Breaking strength/ (g/d)	4.0-5.0	5.0-6.0	Variable
Half-life in 37°C normal saline	4-6 months	4-6 months	2-3 months

These thermal properties and degree of crystallinity are dependent on different factors such as the molecular weight, purity of the polymer, crystallization kinetics and melting behavior of the polymer. [15]

The semicrystalline PLA is often preferred to the amorphous polymer, especially when higher mechanical properties are desired. Brittle PLA can be modified by importing flexibility into the chains with polymers for example by copolymerizing lactide with ε-caprolactone. [17, 18]

Another common polyester, PGA is known for being the simplest linear aliphatic polyester. Glycolide monomer is synthesized by dimerization of glycolic acid. Polymerization happens via ring-opening polymerization, which is the most common method utilized. Ring-opening polymerization yields high-molecular-weight materials with only about 1-3% residual monomer present. Crystallinity of PGA is 45-55 %, so it can be classified as semicrystalline. Common degradation time for pure PGA is from 6 to 12 months. [13] Chemical structure of PGA is presented in Figure 2.

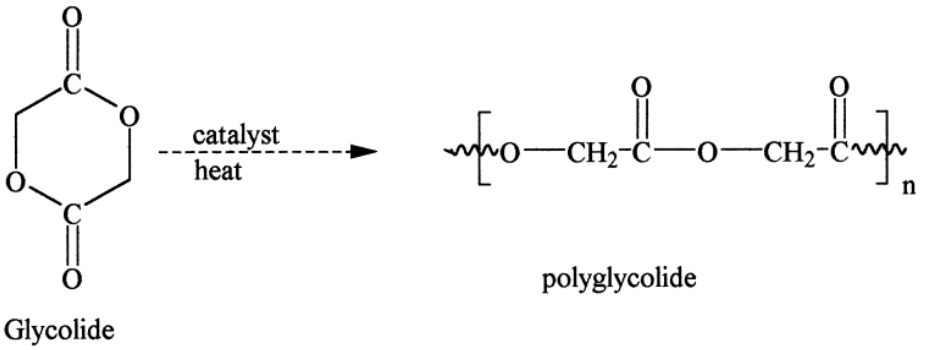

Figure 2. *Chemical structure of PGA. [13]*

PLGA is a copolymer of lactide and glycolide monomers. By copolymerizing these two monomers, it is possible to extend the range of the homopolymer properties. [19, 20, 21] The chemical structure of PLGA is presented in Figure 3.

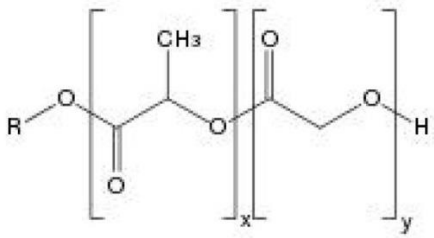

Figure 3. *Chemical structure of PLGA. [8]*

Melting point of PLGA is around 220-225 °C and a glass transition temperature is around 40-60 °C. [22] These thermal properties, which vary with the relation of lactide and glycolide of the polymer, have an effect on the mechanical properties and degradation time.

PLGA is physically strong and highly biocompatible and has tunable mechanical properties. [8] However, these mechanical and degradation properties are not in linear relationship to the homopolymer composition. This is presented in Figure 4, where can be seen for example that, copolymer in 50/50 relation degrades faster than either of these homopolymers. For example PLGA 50/50 degrades in 1-2 months, whereas PLGA 85/15

degradation takes at least 5 to 6 months. [13] These values are influenced by several factors, such as molecular weight of the material.

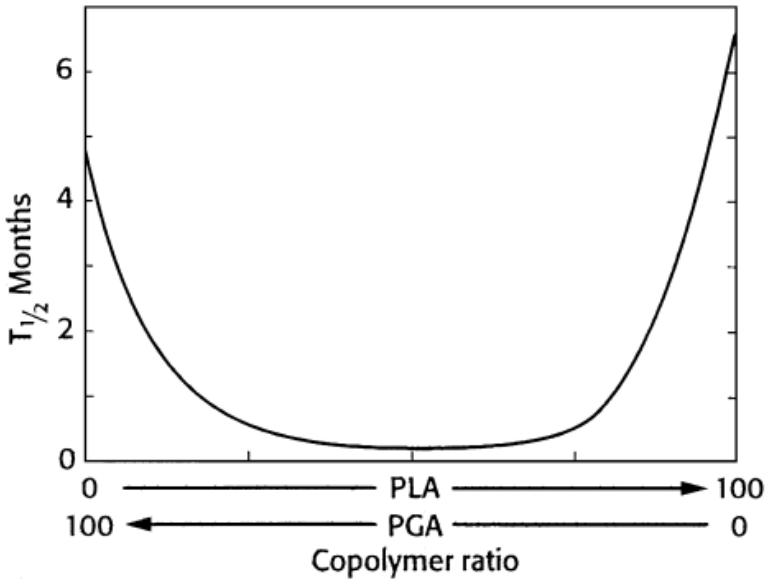

Figure 4. *Half-life of PGA and PLA co- and homopolymers, implanted in rat tissue. [13]*

PLGA has long clinical history as delivery vehicles for drugs, and have been extensively studied to carry proteins and various other macromolecules such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and peptides to the target-site. In addition, it has been used as scaffolds for tissue engineering. [8]

2.1.2 Packing materials

In addition to medical device itself, its packaging is also highly regulated by ASTM and ISO. Essential standards include: ASTM D1585 – Guide for Integrity Testing of Porous Medical Packages, ASTM F2097 – Standard Guide for Design and Evaluation of Primary Flexible Packaging for Medical Products, EN 868 – Packaging materials and systems for medical devices to be sterilized, General requirements and test methods, and ISO 11607 – Packaging for terminally sterilized medical devices. [23]

Package testing documents ensure that packages meet regulations and end-use requirements. Manufacturing processes of the packages must be controlled and validated to ensure consistent performance. [23]

Package materials for implantable medical devices can be made for example from acrylic copolymer and polypropylene (PP). [24] Acrylic copolymers are often used as transparent shell and parts for electrical and mechanical products. It is also used to improve the impact resistance and transparency for high polymer. [25] One of the basic monomers

of the acrylic copolymers is acrylic acid, which chemical structure of acrylic acid is shown in Figure 5.

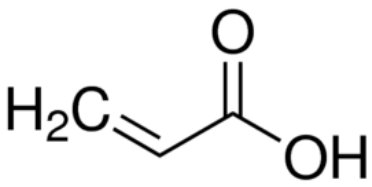

Figure 5. *Chemical structure of acrylic acid. [26]*

Acrylic polymers are slightly hygroscopic, hence, they are able to absorb up to 1.8 m-% moisture when exposed to humid conditions. However, acrylic polymers can be dried easily without raising the temperature in 3 to 4 hours, for example by desiccant dryer. [27]

Polypropylene (PP) is another commonly used polymer in packages for implantable medical devices. PP has a hydrophobic nature [28], due to its chemical structure that is presented in Figure 6. However, it has still ability to absorb small amounts of moisture < 0.01 m-%. [29]

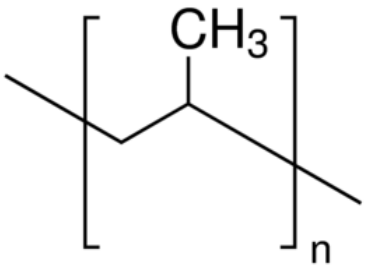

Figure 6. *Chemical structure of polypropylene. [30]*

Polypropylene is often used as a shell for different medical device applications, since it has excellent transparency and chemical resistance, in addition to good physical properties, and printability. PP has a melting point that ranges from 160 to 166 °C [31] PP can be easily processed in different forms of products, whether it would be mouldings, fibres, tape, film or foam. However, it is sensitive to photo-oxidation [32], when the sun light accelerates the rate of oxidation of the polymer chains. [33]

2.2 Moisture absorption

Moisture is defined as the relative mass of liquids, such as water, in the total mass of sample. [34] Moisture absorption is the capacity of the material to absorb moisture from its environment (ambient air, water). [5] Because one of the most used techniques to determine moisture is loss of mass, some other substances are also counted as moisture,

such as, esters, acids and alcohols. Techniques that are based on other than loss of weight -principle, measures water content instead of moisture content, in the material. [34] Most used techniques to determine moisture/water content are introduced in Section 2.2.4.

Water absorption in solid materials starts when they are placed in a humid atmosphere and it continues until the process reaches the moisture equilibrium state. However, in some cases, the ubiquitous latent heat effects may complicate the diffusivity of the water molecules, why the temperature reaches the equilibrium much earlier than the moisture concentration does. [35]

Materials can be classified in two different groups: hygroscopic and non-hygroscopic. Hygroscopic materials have a strong affinity to attract moisture into their molecular structure if exposed to ambient air. Moisture absorption continues until the water vapor pressure within the material increases to equal as the vapor pressure surrounding the material, in other words, when there is moisture equilibrium. [36] Non-hygroscopic material can only collect moisture on its surface. So it is easy to dehumidify by hot air, since there is no internal moisture. [37] In addition, there are several factors that have effect on water absorption, such as, polymer type, used additives, temperature, humidity and exposure time. [5]

Moisture absorption in dependency to time and relative humidity (RH) for PLGA plates according to Park and Kang [2] is presented in Figure 7. It shows the amount of absorbed moisture by PLGA plate at 40 ℃ and in 20 %, 50 % and 80 % RH.

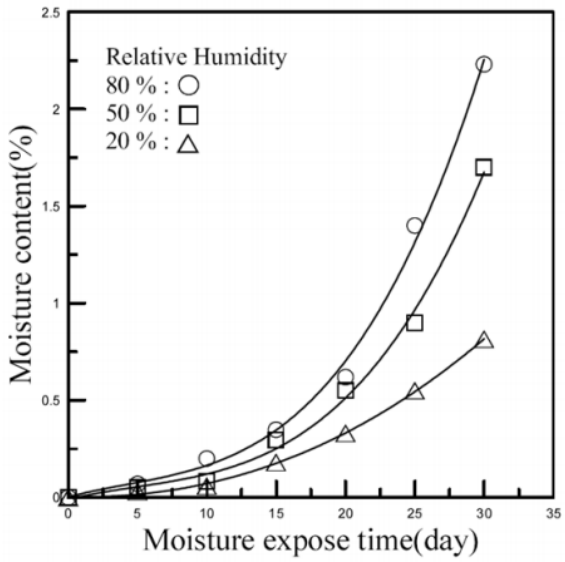

Figure 7. *Absorption of moisture in PLGA plate in 40* ℃ *and in three different relative humidity (20 %, 50 % and 80 % RH). [2]*

As shown in Figure 7, absorbed moisture increases exponentially with exposed time. However, in the beginning (1-5 days) the moisture absorption rate stays rather low. This could be explained by high polymer hydrophobic hydration. [2]

2.2.1 Fick's law

Adolf Fick developed the Fick's law that explains the water vapour diffusion of bound water and interstitial water in selected material. [38] Fick's law is usually presented in the following form:

$$J = -D.\nabla x \tag{1}$$

where, J is the flow of the water and ∇ is the gradient operator. The first Fick's law requires that the flow is proportional to the concentration gradient [38]:

$$J = -D_e \frac{\partial M}{\partial x} \tag{2}$$

where, D_e is the diffusion coefficient. In the second law of Fick the temporal dependence is taken into account [38], when the equation can be said in following form:

$$\frac{\partial C}{\partial t} = \frac{\partial}{\partial x}(D_e \frac{\partial C}{\partial x}) \tag{3}$$

$$\frac{\partial C}{\partial t} = D_e \frac{\partial^2 C}{\partial^2 x} \tag{4}$$

In these equations, C is the concentration. With these equations the movement of the water molecules in the selected material can predicted.

2.2.2 Hydrolysis

Polylactides and polyglycolides are both hygroscopic materials that degrade via hydrolysis by bulk erosion shown in Figure 8, such as their copolymer PLGA. However, some studies suggest that enzymatic degradation may play some minor role in the degradation of PLGA. [39]

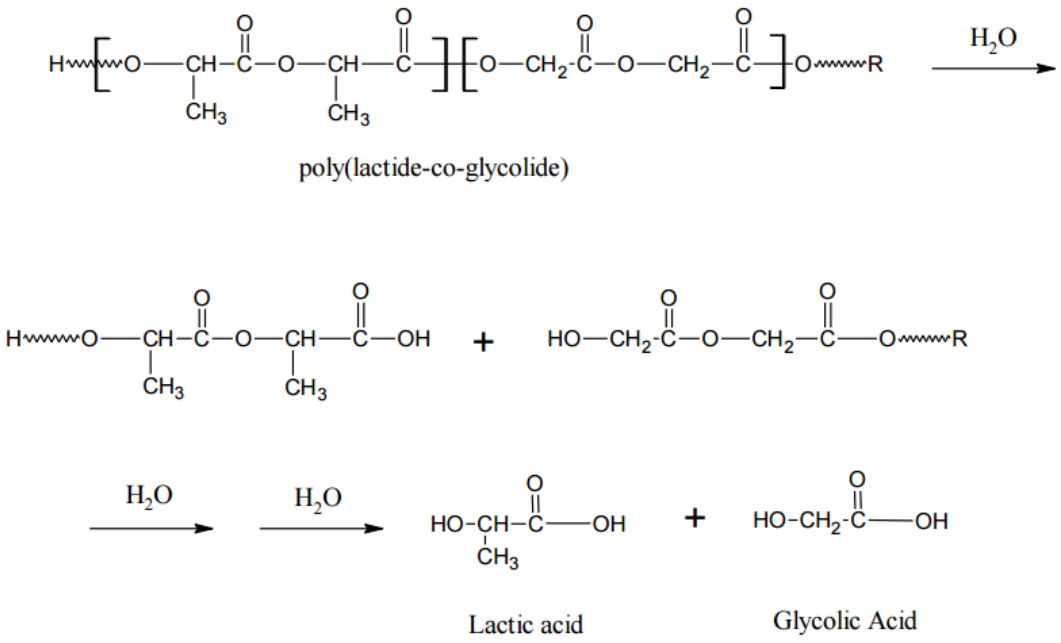

Figure 8. *Degradation of PLGA via hydrolysis. [40]*

Bulk erosion occurs when water penetrates to the entire material and degrades entire material simultaneously. The by-products of the degradation at the surface are rapidly dissolved in the surrounding environment and removed from the bulk polymer. Bulk erosion is presented in Figure 9. [8]

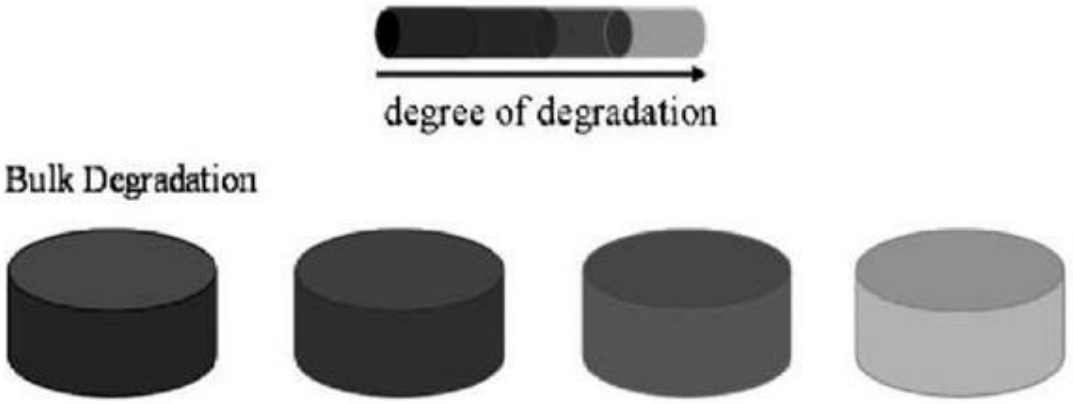

Figure 9. *Bulk degradation. [41]*

The hydrolysis starts with water uptake, where water penetrates into the bulk of the device, and attacks to the chemical bonds in the amorphous phase and cuts long polymer chains into shorter, ultimately hydrolyzed water-soluble fragments. Due to hydrolysis in the amorphous phases, molecular weight starts to decrease rapidly. In this phase, the matrix of the device is held together by the crystalline regions, why there is no loss in physical properties. However, reduction in physical properties is followed as water

begins to fragment the crystalline regions. The final phase in the polymer hydrolysis is rapid loss of polymer mass, as is shown in Figure 10. [13]

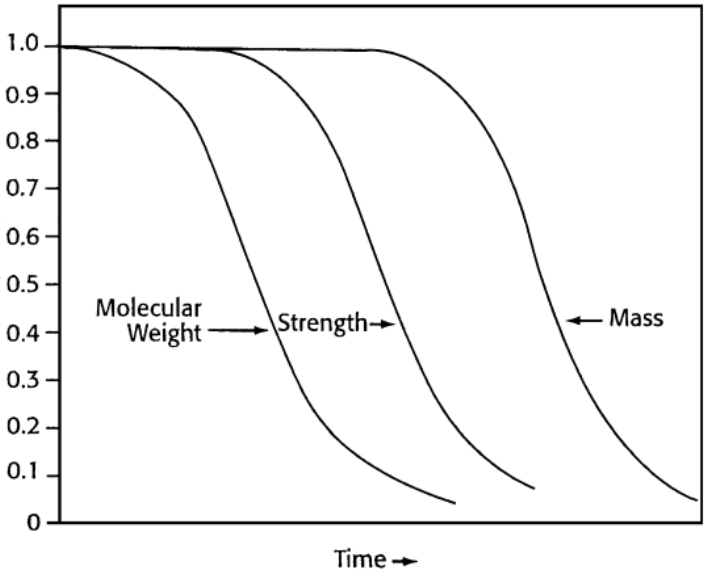

Figure 10. *Decreasing properties during polymer degradation. [13]*

Hydrolysis can be emphasized by different factors. Naturally the relation between PLA and PGA is crucial for all of the properties of the product, but also other factors, including microstructure, part geometry, thickness, vascularization of the location and stress may have an important role in degradation rate. Areas with poor vascularization are not suitable for large implants, because they may degrade faster, and overwhelm the body, by building up acidic by-products, which leads even faster degradation and may cause adverse tissue reactions. If the implant is under stress, it may degrade faster because it forms microcracks, which expose more surface to water absorption, which emphasizes the degradation via hydrolysis. In addition to these, the presence of excess monomers in the material may catalyze the hydrolysis and act as plasticizers, which change the mechanical properties of the material. [13]

The degree of crystallinity of the PLGA has major effect on different properties such as mechanical properties, swelling behavior, capacity to undergo hydrolysis and subsequently biodegradation rate of the polymer. When crystalline glycolide is co-polymerized with lactide, the degree of crystallinity of PLGA reduces and as a result the rate of hydration and hydrolysis are increased. Physical properties of PLGA have been shown to depend on several factors, including the initial molecular weight, lactide-glycolide ratio, the size of the device, shape of the surface and temperature. [7]

The hydrolysis of lactic acid based polymers can be undesired at certain circumstances, but beneficial in other instances, for example biodegradable medical devices or com-

postable packages. [13] However, for example during processing and storaging, moisture content of PLA and PGA have to be taken to account because PLA and PGA are hydrolytically unstable, hence prone to undergo degradation. Especially PGA, which is even more sensitive to hydrolytic degradation due to its chemical structure. [13]

2.2.3 Effects of moisture on polymer

Moisture has an effect on different material properties, such as the shelf life, dimensional stability, mechanical strength and electrical conductivity and the dielectric loss factor. [5] All of these properties affect in the quality of products. Monitoring of moisture content is therefore important during the production and after it. Most materials have optimum moisture content for obtaining the best possible processing results and achieving maximum quality. [5, 14]

During processing hygroscopic material absorbs moisture if present, which leads to unwanted hydrolysis during the processing. Hydrolysis makes the long polymer chains shorter, what is not desirable quality of the product. Since short chains may result in poor quality molding. [42] If the material is not dried, the moisture will react with the molten polymer, due to the higher temperatures during processing phases, resulting in a loss of molecular weight. This loss leads to lowered mechanical properties such as reduced tensile and impact strengths. [43]

Processes, where the temperature is rising relatively high, such as extrusion, are the most challenging parts of the production phases for moisture. To avoid the loss of the molecular weight during these processes with high temperature, acceptable moisture rate for material before the processes should be less than 0.02 m-% [44, p. 158, 45]. Processes with only slightly risen temperature (above T_g), the temperature will not go as high why little more moisture is allowed. [46]

Physical properties (glass transition temperature, molecular weight, etc.) of PLGA are influenced by absorbed moisture in the material. The T_g of the PLGA copolymers are reported to reduce with a decrease of lactide content in the copolymer composition and with a decrease in molecular weight. [47] The decrease of the T_g have been noticed also in other polyesters. [13] The Gordon-Taylor equation (Formula 5) expresses the glass transition temperature in relation to water content in PLGA within narrow range of moisture content and limited polymer degradation. [2]

$$T_g = \frac{w_1 T_{g_1} + \chi w_2 T_{g_2}}{w_1 + w_2} \text{ [48]} \quad (5)$$

where w_1 and w_2 are the weight fraction of two components, T_{g_1} and T_{g_2} are respective T_g of the neat component and χ is a parameter related to the strength of the interaction between the two polymers. [48]

Moisture absorption can influence also to metal-to-plastic contact regions. In these regions after packing the product, moisture vaporizes during the rapid heating and generates pressure along. Differences between the coefficients of thermal expansion of the two materials (plastic/metal) can cause loss of adhesion, swelling, and even cracking. [49]

2.2.4 Moisture analysis methods

There are several methods to determine the moisture content in the material. They are classified in two different categories, based on their determination methods. Methods, that determine moisture content, such as moisture analyzer, drying oven, and thermogravimetry, and methods that determine water content, such as Karl Fischer titration, spectroscopy, and chromatography.

The most traditional method to determine the moisture content is to weight the sample to get wet mass, m_{wet}, dry the sample for example in vacuum, and then weight the sample again to get the dry mass, m_{dry}. The difference with these values tells how much there was moisture in the sample and then the mass procent can be calculated by dividing the difference with the dry mass as presented in Formula 6.

$$Absorbed\ moisture\ content\ (\%) = c \times 100\ \% = \frac{m_{wet} - m_{dry}}{m_{dry}} \times 100\ \% \qquad (6)$$

Another common methods are Karl Fischer titration and Halogen moisture analyzer. Karl Fischer is used to determine the water content of samples. It can provide more accurate results than simple weight loss –method, since in the weight loss method volatiles other than water are interpreterred as moisture content, that can easily be mixed with water content. However, there are several parameters that have to be precisely determined, so that the apparatus works as expected.

In the Karl Fischer titration method a small weighed sample is placed into the drying oven at a predetermined temperature for a predetermined period of time. These parameters has to be exactly right, because if the temperature is too low or the retention time too short, all of the water may not be removed, and if the temperature value is too high polymer can start to degrade and actually generate water. [34]

Karl Fischer titration is a two phase reaction. In the first reaction, an alcohol, sulfur dioxide (SO_2) and a base (RN) react to form an alkylsulfite intermediate, which is presented in Formula 7:

$$CH_3OH + SO_2 + RN \leftrightarrow [RNH]SO_3CH_3 \quad (7)$$

In the next reaction, which is shown in Formula 8, the alkylsulfite, which was formed in the first reaction, reacts with iodine (I_2) and the water from the sample:

$$[RNH]SO_3CH_3 + I_2 + H_2O + 2RN \leftrightarrow [RNH]SO_4CH_3 + 2[RNH]I \quad (8)$$

Because amounts of water and I_2 are consumed in equimolar amounts (see Formula 8), the amount of water can be calculated from the amount of used I_2. [50]

There are two methods used to perform the Karl Fischer titration test, volumetric and coulometric titration. The difference between them is primarily in the way the I_2 is generated. In volumetric titration, the I_2 is included with the reagents, when in coulometric titration, the I_2 is generated at an electrode. [34] Comparison of these two methods are presented in Table 2.

Table 2. *Comparison of coulometric and volumetric Karl Fisher titration. [51]*

Property	**Coulometry**	**Volumetry**
Water amount and sample amount	Small water amount Small sample amounts	Medium and large water amounts Adapted sample amount
Sample types	Liquid Gaseous Solid samples with oven	Solid Liquid
Sample addition and preparation	Direct with syringe Gas inlet with oven External extraction Solid samples are evaporated with an oven	Direct with syringe Solid samples are added directly Sample preparation with homogenisator Working at higher temperature
Working method	Very fast Very simple	Fast Simple
Working range	µg range 10 µg up to 5 mg water	mg range 200 µg up to 50 mg water
Trueness	Pretty good for small water amounts	Pretty good for water amounts > 5 mg water
Reproducibility	Typical reproducibility standard deviation of approximately 1 % for water > 400 µg	Typical reproducibility standard deviation of approximately 1 % for water > 5 mg

Moisture analyzer determines the moisture content in the material, so it includes other volatiles as well, in addition to water. [52] The Halogen Moisture Analyzer works by thermogravimetric principle, so it measures the start weight of the sample, and then a halogen radiator dries it while the sample weight is continually recorded. The total loss in weight is compared to the initial weight and finally can be interpreted as moisture content. [53]

Drying occurs via further developed infrared drying method by halogen radiator. The heating element consists of a glass pipe filled with halogen gas. Because, the mass of the halogen radiator is relatively low, halogen moisture analyzer absorbs the thermal radiation from the halogen lamp quickly, which results outstanding controllability, compared with conventional infrared radiator. In addition to this there is a gold-plated reflector, which ensures an optimum, evenly distributed thermal radiation over the entire sample surface. This is important to obtain repeatable results. [53] The basic principle in halogen radiator is presented in Figure 11.

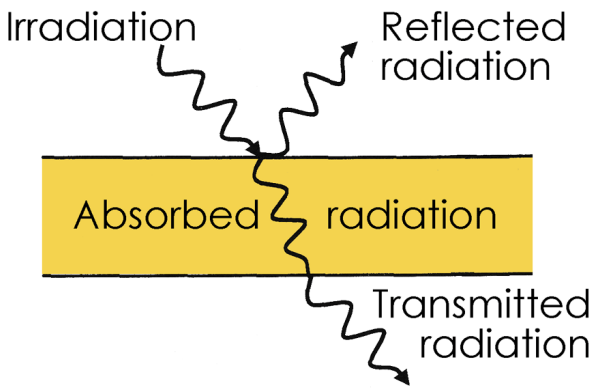

Figure 11. *Absorption of the IR radiation. [54]*

The determination of the measurement is automatically ended and the result displayed by the switch-off criterion (AK). There are several types of AK, but two most used ones are: A time-controlled form of switching off or the decrease in weight per time unit. Standard setting for switch-off criterion is 1 mg/50 s, based on decrease in weight per time unit. [53]

2.3 Drying

Drying is used to minimize the problems that moisture cause in the polymer during processing. Especially hygroscopic resins have a strong affinity to absorb moisture and if they are not dried prior to processing, the physical properties of that material may be drastically affected. [55]

For sensitive materials all of the drying method are not suitable. For example hot temperatures (above the glass transition temperature) may lead to fusing in most of the amorphous polymer. [13]

There are various types of drying technologies available, including hot air dryer, desiccant wheel, vacuum, compressed air dryer, and membrane dryer. Dryer selection has to be selected by the performance requirement of the process, as well as price and other features wanted. [55]

Every material aims to equilibrium moisture content. This means that water moves from regions with higher moisture content to regions with lower values. [56] This phenomenon is explained by the second law of thermodynamics. [57]

2.3.1 Drying parameters

Drying has four important parameters: heat, dew point, drying time and air flow. Heat is the most important drying parameter because if there is no heat, the material will not release moisture inside the material. So heat is the driving force in drying. It causes the molecules to move around more intensely, weakening the forces between the water molecules and the polymer chains. Dew point is the temperature, at which the water vapor of the air condenses into liquid water at the same rate as it evaporates, at constant barometric pressure. [37]

Third parameter is drying time which is case-specific in different materials. The drying time is the time requested at a particular dew point and temperature to dry the material to a set degree of residual humidity. Too long drying time can cause a degradation of the polymer and decrease of the physical and other properties and wasting energy. [58] Fourth parameter is the airflow. It carries the heat or dry heated air to the material and carries moisture away from the material. [37]

2.3.2 Drying methods

There are several different dryers for polymer material with different principles. Hot-air dryers are the oldest, simplest, and least expensive drying method. Hot air dryer is suitable when surface moisture is needed to remove from non-hygroscopic resins. Generally, dryers are able to reduce the residual humidity from 0.1 to 0.02 % and in special cases even up to 0.002 %. Drying temperatures are typically between 65 °C to 180 °C. [58] Its working principle is very simple. When a hot, and dry air surrounds a wet hygroscopic material, the moisture within the material begins to migrate toward outside the material, in where is lower vapor pressure until it has reached moisture equilibrium with the surrounding dry conditions. [36] Hot-air unit consists of a heater blower unit connected to the drying hopper. Surrounding air is drawn through a filter into a heater blower unit and circulated up through the material in the hopper, carrying surface moisture up and out of the top of the hopper. [59] The basic principle behind the hot air dryer is presented in Figure 12.

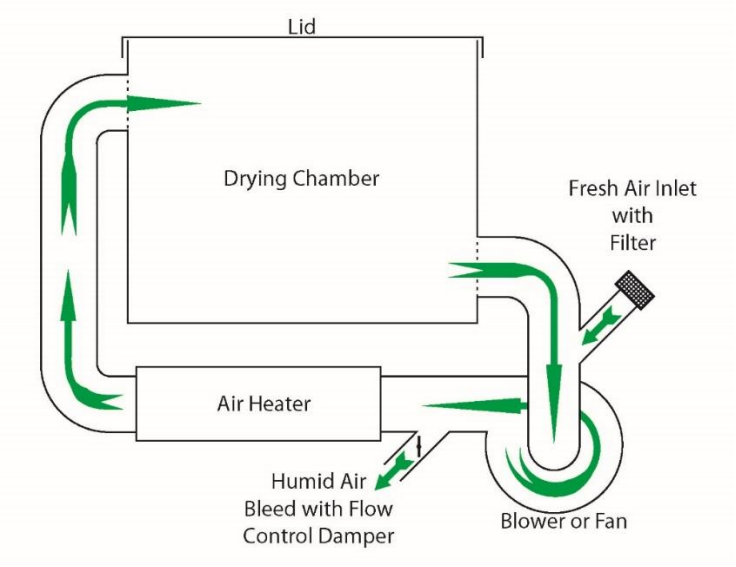

Figure 12. *Basic principle behind the hot air dryer. [60]*

There are three basic styles of desiccant dryers: twin-tower, carousel designs with two or more desiccant beds, or rotating wheel desiccants. Wheel desiccants are nowadays the most used desiccant dryers, because of their properties, such as less maintenance, and lower energy usage. They are well suitable option for drying any hygroscopic resin. Wheel desiccant unit is presented in Figure 13. [61]

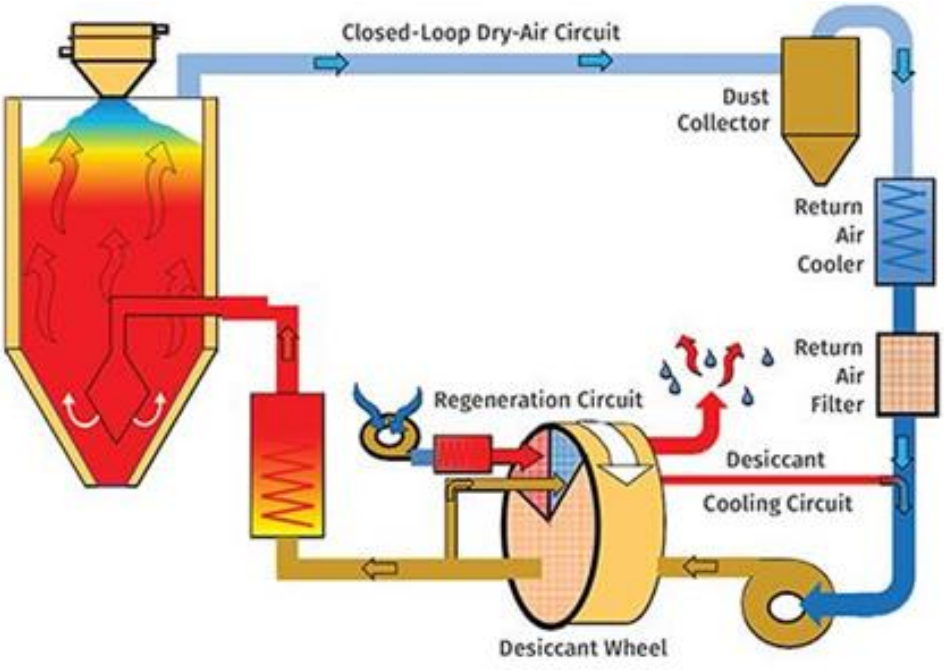

Figure 13. *Basic principle behind the desiccant wheel dryer. [61]*

This continuous process ensures that dry desiccant is always available, and therefore the efficiency of the process increases, power consumption decreases, and -40 °C dew point is guaranteed. [61]

In compressed-air dryers, compressed air re-enters atmospheric pressure with less moisture than it contained prior to compression. This means that the dew point of the de-compressed air is reduced by about 40-50 °C which cause multiple advantages, such as providing the ability to use compressed air for drying material in a hopper. The compressed air is also filtered, so it can remove course amounts of moisture and dirt. [62]

Compressed air dryers are simple and relatively maintenance free but they have some big disadvantages. In the process air never reaches a -40 ˚C dew point. In addition to this, dew point varies with the surrounding conditions, such as humidity. Also the energy usage is relatively high compared to other dryer types, since the use about 3 times as much compressed air as membrane dryers. [62]

Membrane dryer include membrane in the drying unit in addition to compressed air dryer. However, there is a big difference between a compressed air dryer and a compressed air dryer with a membrane. [63] A membrane enhances the compressed air dryer by enabling lower dew point air, why the drying capability of it is better compared to conventional compressed air dryer. [64] The principle behind the membrane dryer is presented in Figure 14. [63]

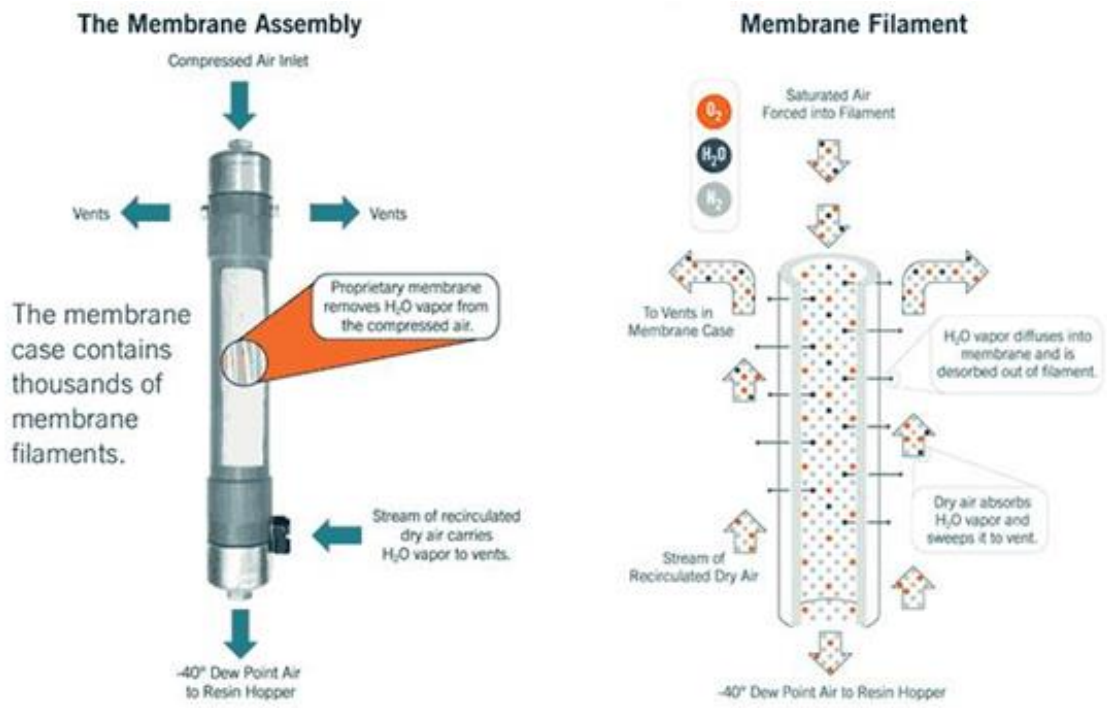

Figure 14. *Principle of the membrane dryer. [63]*

Since the membrane removes the moisture from the air, the dew point of the dryer can achieve even -40 °C, hence the H_2O can be removed by the membrane. [64]

Vacuum drying reduces the boiling point of water, and literally pulling the water vapor from the material. [65] Vacuum dryers use a multi-stage process, which is shown in Figure 15.

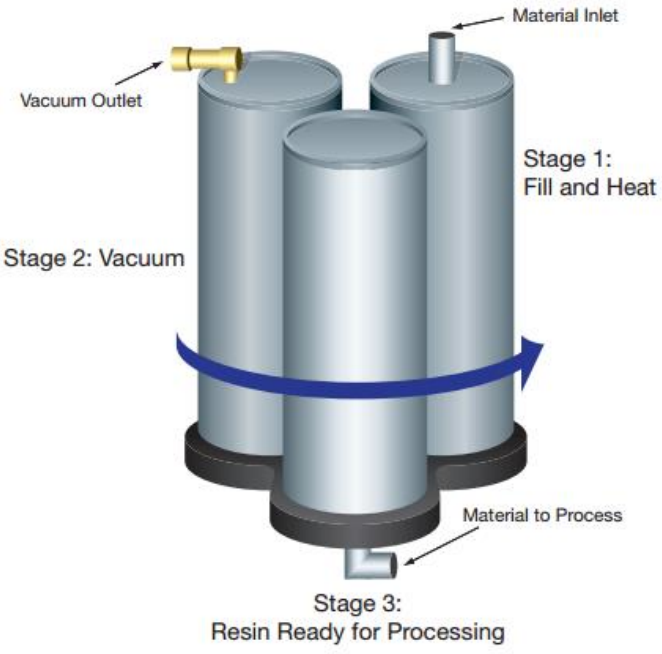

Figure 15. *Vacuum dryer. [66]*

Plastic material is contained in a stainless steel sealed container. At the first, material is loaded into the dryer and possibly heated to allow the molecular release of moisture. In the second phase, the material is repeatedly subjected to a strong vacuum, in a way that moisture is drawn away from the material. The dried material is discharged at the last phase. [67] The biggest advantage for the material properties is, that in vacuum dryers, material is in heated environment for only a little time or not at all, so there may only be little material degradation. [66] There are also other advantages, such as faster and less energy consumptive drying process. [67]

2.3.3 Dehydration gas

The reason why dehumidifying gas is preferably needed at least in vacuum ovens is that, when these vacuumed samples were taken out from the vacuum oven, the surface of the samples should have been theoretically empty at this point due to the vacuum. When these samples were exposed to ambient air, the samples absorb everything, including gaseous water molecules, onto the surface, since the surface is available and all molecules move towards the moisture equilibrium. [56] This is the case when there is

not any dehumidifying gas inside the vacuum oven. However, when there is nitrogen, argon, CDA or something else dry gas inside the vacuum oven the samples are surrounded by some molecules of these gases. And therefore the surfaces of the samples are at least nearly full of the molecules, which will complicate and slow down the water intake at the surfaces.

In desiccators, either compressed dry air, or dry nitrogen are often used as a dehumidifying gas. There are also other gases for that purpose available, such as dry argon, but the disadvantages are greater than the benefits. Dry argon gas is heavier than air and nitrogen, so it would be easier to manipulate, but it is relatively expensive, compared to nitrogen and especially to compressed air. [68]

In many cases, a steady stream of gas is used to evaporate the solvent. Pure, dry nitrogen is commonly used, as it is a relatively inert gas, hence it does not interact with the material. However, since its chemical activity, it also does not react with water molecules. [69] This is due its chemical structure that is presented in Figure 16.

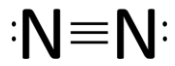

Figure 16. *Chemical structure of the gaseous nitrogen molecule.*

Since nitrogen has triple bond between nitrogen atoms and both nitrogen atoms has 2 electrons in their outermost surface the molecule stays nearly inert. Because nitrogen does not react with the free water molecules in the atmosphere, it means that it does not carry the moisture out from the surroundings of the material in an effective way. The drying process originates only from the equilibrium of the gaseous water molecules. [56] Water molecules move towards the equilibrium when water molecules move out from the material as illustrated in Figure 17.

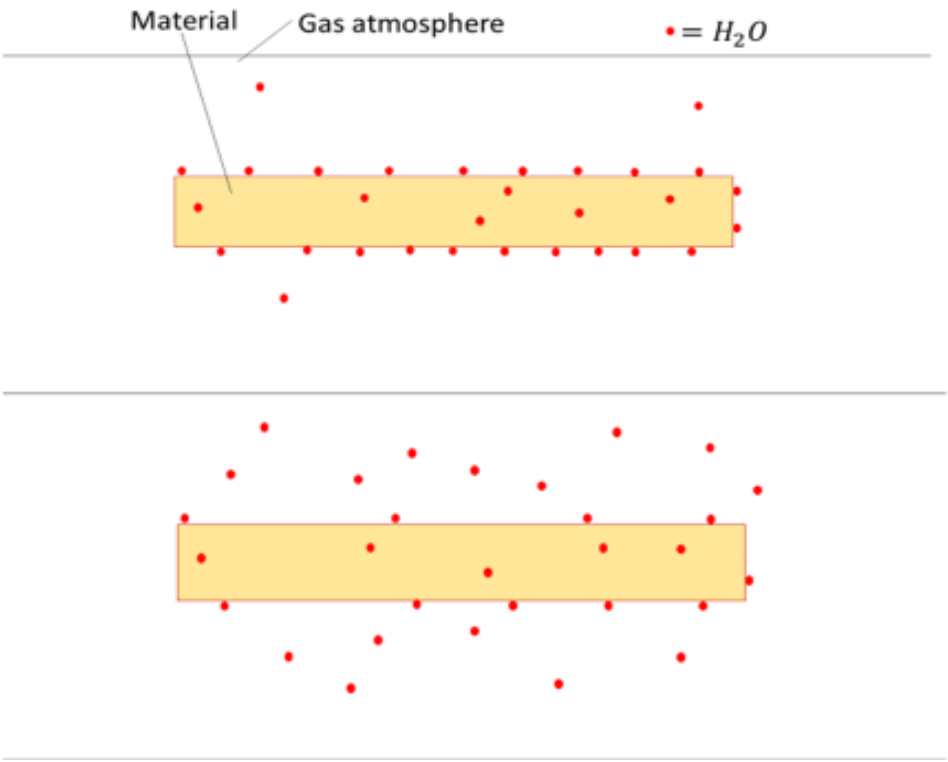

Figure 17. *Water molecules move towards the equilibrium in gas atmosphere.*

After the water molecules are distributed in the atmosphere, nitrogen flow pushes these water molecules away from material and the movements of the water molecules towards the equilibrium start again. Without the nitrogen flow, gaseous water molecules stay in the atmosphere and can easily react again with the material. Using nitrogen offers a number of advantages for drying operations. Nitrogen provides an inert atmosphere that is ideal solution for long-term preservation once the drying process is completed. This environment reduces oxidation and no purge is required after drying is complete. [50] In order to achieve faster evaporation process, nitrogen can be heated as well as air, which provides even more effective drying, [70] since the relative humidity is dictated by air temperature. [71]

In contrast to nitrogen, air differs in many aspects. Air consists of nitrogen (~78 %), oxygen (~21 %),argon (~1 %) and several different gases such as carbon dioxide, nitrous oxides, methane, and ozone. [72] Since the oxygen (O_2) in the air, can easily binds water vapor from the atmosphere and also from the material. [73] Oxygen is more reactive than nitrogen molecule since oxygen has only double bond between the oxygen atoms why in both atoms there are 4 electrons that are ready to react with water molecules. The chemical structure can be seen from the Figure 18. Due to its chemical structure moisture wants to react with oxygen and hence the drying process of the material does not rely only on equilibrium of the water molecules.

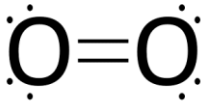

Figure 18. *Chemical structure of gaseous oxygen molecule.*

Relative humidity is the amount of water vapor that the air can hold. Since nitrogen has a dew point of -70 ℃ [74], that is colder than the dew point of CDA (-40 ℃ [62]), nitrogen can hold more moisture in cold circumstances. However, since one requiment of the drying system is the gas flow, the amount that gas can hold water molecules is irrelevant, since all of the moist gas is replaced with dry gas.

Oxygen may have more efficient transportation of the water molecules due to its chemical activity, since oxygen is very reactive with the water molecules. However, oxygen have also its drawbacks, such as its ability to react with the polymer chain and in that way cut the chain. This reaction can be considered as thermo- or photo-oxidative degradation. [75] In these degradation processes, the initiators of the degradation are heat and light.

3 AIMS OF THE STUDY

The aim of this work was to study moisture absorption of PLGA. During this project, the production and its subprocesses were focused from a moisture perspective and alternative more cost-effective methods were examined and experimented.

The main questions of this thesis are:

1) How fast does the PLGA absorb the moisture?
2) Which gas is the best/most cost-effective option for the dehumidifying gas: nitrogen or CDA?
   - How the gas affects in the mass of the sample?
   - How fast gases are able to dry?

4 MATERIALS

Samples were selected in a way that they would describe the behavior of the “a worst case” scenario. Selected materials are presented in Table 3.

Table 3. *Used samples.*

ID	Material	Diameter (mm)	Length (mm)	Shape
1	PLGA	4.10 ± 0.10	25 ± 1	Process phase 1 sample
2	PLGA	2.15 ± 0.05	25 ± 1	Process phase 2 sample
3	PLGA	1.60 ± 0.10	25 ± 1	Process phase 2 sample

The test samples are presented in Figure 19 and Figure 20.

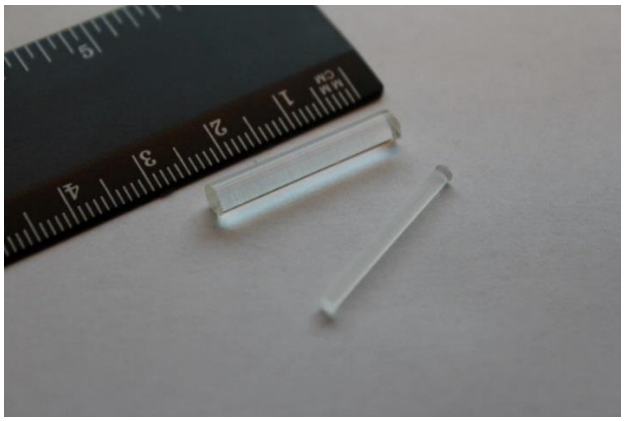

Figure 19. *PLGA samples with 4.1 mm and 2.15 mm diameter.*

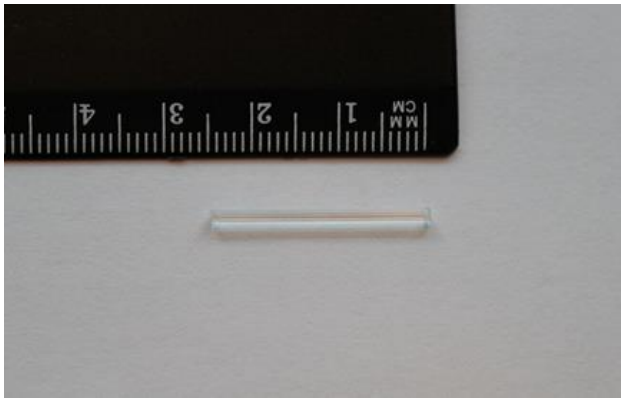

Figure 20. *PLGA samples with 1.60 mm diameter.*

In addition to these, two different gases were compared. These gases are presented in Table 4.

Table 4. *Detailed information about the used gases.*

Gas	**Pressure (bar)**
Nitrogen	0.1
CDA	0.1
CDA	1

These gases were pre-dried and purified so that the purity of the gases should theoretically be nearly 100 %. The moisture contents of these gases were confirmed, and they were approved to dry gases for these tests series.

5 METHODS

5.1 Absorption rate of PLGA in standard conditions

Measurements were done according to standard ISO 62:2008. Three 4.10 mm by their diameters (process phase 1) and three 2.15 mm by their diameters (process phase 2) samples were made and cut beforehand (means of measuring the dimensions of the test samples to an accuracy of ± 0.1 mm) and stored in nitrogen atmosphere. This number of samples was chosen according to ISO 62:2008 [76], which says that, by having three or more parallel samples the results will have remarkable statistic value.

From nitrogen atmosphere, samples were transported in aluminum foil bag and placed into beaker, that is placed in the vacuum oven (WBT Binder VD115, vacuum pump: Trivac D 4 B), in a room temperature (to nearly 0×100 mbar), where the samples were put for 24 ± 1 h. After the vacuum drying, the samples were put into desiccator (VWR Prolabo, Chameleon® Silica gel 2-6 mm) during the transportation, and weighted by balance (TEOPAL, Precisa 240A), which has an accuracy of ± 0.1 mg. After the weighting, the test number one samples were moved into environmental chamber (OZONE GENTLE, Model: PR-1KPH) in individual stainless steel cages in 5 liters beaker, whereas the test samples in test number two were moved into environmental chamber (OZONE GENTLE, Model: PR-1KPH) in individual Petri dishes. Stainless steel cages for test number one were made, since the samples should be under the water during the 24 hours. Used stainless steel cages are presented in Figure 21.

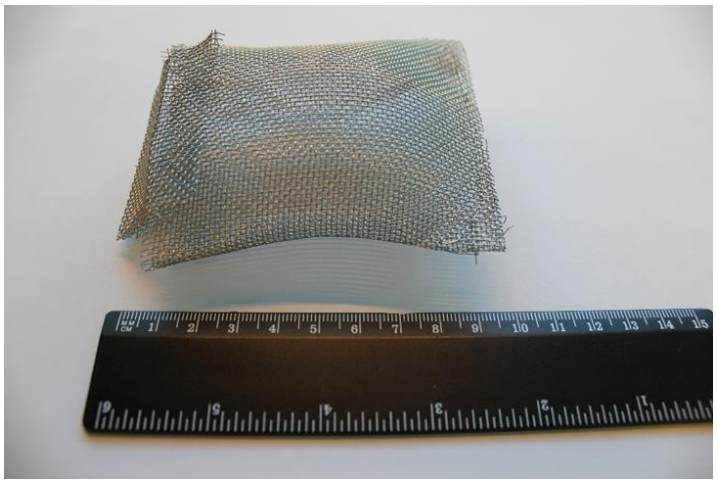

Figure 21. *Stainless steel cages, made from stainless steel web and wire.*

In the cabinet the atmosphere conditions were following: In standard conditions, first test had temperature 23 ℃ and relative humidity 100 % (300 ml of distilled water for

every sample to prevent any extraction product from becoming excessively concentrated in the water during the tests), whereas in the second test, temperature was the same 23 ℃, but the relative humidity was set 50 %. After 24 hours, samples were weighted during under 1 minute. From the weighted masses, absorbed moisture contents were calculated by the Formula 6. This gave the absorbed moisture as a m-%.

From Fick's law the diffusion coefficient can be calculated. There is a following connection between moisture content c and moisture content at saturation c_s:

$$\frac{c}{c_s} \leq 0.51 \tag{9}$$

This formula can be said also in different form:

$$\frac{D\pi^2 t}{d^2} \leq 0.50 \tag{10}$$

where t is the duration of immersion of the test sample in the water or humid air, in seconds, D is the diffusion coefficient and d is the thickness of the test sample, in millimeters. [76]

5.2 Effects of the dehumidifying gas on the sample masses

Two gases were studied: dry compressed air and dry nitrogen gas. The moisture content in the sample were measured with weighting principle (Formula 6) in laboratory, by using analytical scale (Sartorius CP225D), which has approximately $\pm$ 0.1 mg error limit. Samples were weighted in once a day during the first 6 days. After those 6 days, masses were weighted again after four days and then vacuum dried and weighted the dry masses to specify the (difference between) moisture contents of these two sample groups. Measured weights were recorded and are presented in 0.

The vacuum drying was performed by Memmert GmbH, Memmert VO400 vacuum oven at 25 ℃, with nitrogen cycles. The drying process lasted approximately for 19 hours.

Experiment was performed with container with a gas flow in a clean room. Two tubes were used. Schematic figure of the testing arrangements is presented in Figure 22.

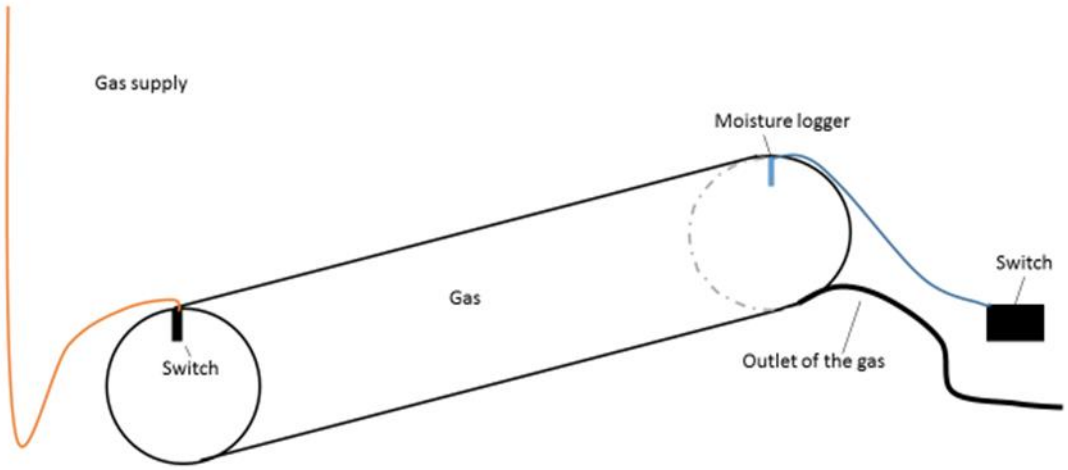

Figure 22. *Schematic picture of the test arrangements.*

There was a gas flow with small overpressure ~1 bar of CDA and ~0.1 bar of nitrogen, that pushes the moist gas away from another end of the tube, whenever the relative humidity inside the tube exceed 7 %. When the RH of the tube was greater than 7 %, electrical switch opened and because of the small overpressure, moist gas was removed from the tube and replaced by dry gas until the RH is 4 % or smaller. In both tubes there were moisture loggers that monitored the relative humidity every 3 hours.

5.3 The recovery time of the gas

The recovery time of the gas humidity inside the tube after opening the cap was also studied. The cap was kept open for three minutes and then closed and waited until the RH inside the tube stabilizes. The RH was measured by Honeywell Sensing and Control:HIH-4000 and interpreted by a custom made control software. The time that gases took to dry the tube is compared in 6.3.

6 RESULTS

6.1 Absorption rate in standard conditions

Absorption rate of the selected PLGA samples were measured, according ISO 62 test. Results of the tests and calculated values according to Formula 6 are shown in Table 5 and Table 6. Samples 1, 2 and 3 are process phase 2 samples and samples 4, 5 and 6 are process phase 1 samples.

Table 5. *Results and calculated values after 23 ℃ and 100 % RH conditions.*

	Sample	Dry mass (g)	Wet mass (g)	Mass difference (g)	Absorbed moisture (m-%)
Processing	1	0.1037	0.1065	0.0028	2.7001
process 2	2	0.1046	0.1061	0.0015	1.4340
sample	3	0.1043	0.1068	0.0025	2.3969
Processing	4	0.4310	0.4330	0.0020	0.4640
process 1	5	0.4149	0.4170	0.0021	0.5061
sample	6	0.4117	0.4140	0.0023	0.5587

Table 6. *Results and calculated values after 23 ℃ and 50 % RH conditions.*

	Sample	Dry mass (g)	Wet mass (g)	Mass difference (g)	Absorbed moisture (m-%)
Processing	1	0.1034	0.1044	0.0010	0.9671
process 2	2	0.1033	0.1042	0.0009	0.8712
sample	3	0.1038	0.1042	0.0004	0.3854
Processing	4	0.4145	0.4150	0.0005	0.1206
process 1	5	0.4281	0.4286	0.0005	0.1168
sample	6	0.4247	0.4258	0.0011	0.2590

The amount of absorbed moisture is presented in relation to time in these two different conditions are presented in Figure 23.

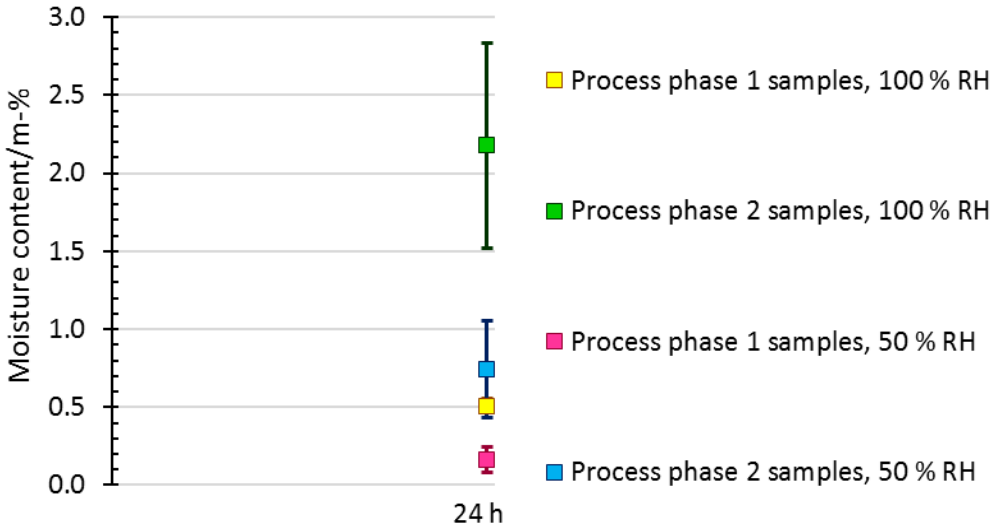

Figure 23. *Absorption rate at 23 ℃ and 100 % RH and 50 % RH.*

The average value of the water absorption in 100 % RH and at 23 ℃ is 0.51 ± 0.05 m-% for process phase 1 samples and 2.18 ± 0.67 m-% for the process phase 2 samples. In 50 % RH and at 23 ℃ the average value of the water absorption is 0.17 ± 0.08 m-% for process phase 1 samples and 0.74 ± 0.31 m-% for the process phase 2 samples.

Since it is known how much moisture have effect on material properties, it might be beneficial to calculate also how long it takes that billets absorb too much moisture before different heat requiring processes. Hence, in addition to the previous results, time, when the moisture contents would reach the critical moisture contents 0.02 m-% (the value that should not be pass during high temperature processing) and 0.05 m-% (the maximum moisture content value for the product) were calculated from the equations of the lines. As an example the time when the moisture reaches 0.02 m-% for the sample 1 at the 100 % RH is calculated:

$$y = 0.1125x$$

$$x = \frac{y}{0.1125}$$

$$x = \frac{0.02}{0.1125} = 0.17777 \ldots \text{h} \approx 10.7 \text{ min}$$

The results are shown in Table 7 and Table 8.

Table 7. *Time, when moisture values would reach 0.02 m-%/0.05 m-% were calculated for every pre-dried samples at 23 ℃ and 100 % RH.*

Specimen	Equation	Time; 0,02 m-% (h:min)	Time; 0,05 m-% (h:min)
1	y=0.1125x	0:11	0:27
2	y=0.0597x	0:20	0:50
3	y=0.0997x	0:12	0:30
4	y=0.0193x	1:02	2:35
5	y=0.0210x	0:57	2:23
6	y=0.0232x	0:52	2:09

Table 8. *Time, when moisture values would reach 0.02 m-%/0.05 m-% were calculated for every samples at 23 ℃ and 50 % RH.*

Specimen	Equation	Time; 0,02 m-% (h:min)	Time; 0,05 m-% (h:min)
1	y=0.0403x	0:30	1:14
2	y=0.0363x	0:33	1:23
3	y=0.0160x	1:15	3:12
4	y=0.0050x	4:00	10:12
5	y=0.0049x	4:05	10:00
6	y=0.0108x	1:51	4:38

Moisture content at saturation c_s is calculated with the Formula 9 for both process phase 1 and process phase 2 samples.

Process phase 1 sample:

$$\frac{c}{c_s} \leq 0.51$$

$$c \leq 0.51 \times c_s$$

$$c_s \geq \frac{c}{0.51}$$

$$c_s \geq \frac{0.00165476254}{0.51}$$

$$c_s \geq 0.0032446 \ldots$$

Process phase 2 sample:

$$c_s \geq \frac{0.00741241078}{0.51}$$

$$c_s \geq 0.0145341 \ldots$$

Diffusion coefficient is calculated with the Formula 10 for both both process phase 1 and process phase 2 samples.

Process phase 1 sample:

$$\frac{D\pi^2 t}{d^2} \leq 0.50$$

$$D\pi^2 t \leq 0.50 \times d^2$$

$$D \leq \frac{0.50 \times d^2}{\pi^2 t}$$

$$D \leq \frac{0.50 \times 4.13\ \text{mm}^2}{\pi^2 \times 86477{,}5\ \text{s}} = 9.9923 \ldots \times 10^{-6}\,\frac{\text{mm}^2}{\text{s}} \approx 9.992 \times 10^{-6}\,\frac{\text{mm}^2}{\text{s}}$$

Process phase 2 sample:

$$D \leq \frac{0.50 \times 2.09\ \text{mm}^2}{\pi^2 \times 86477{,}5\ \text{s}} = 2.5589 \ldots \times 10^{-6}\,\frac{\text{mm}^2}{\text{s}} \approx 2.559 \times 10^{-6}\,\frac{\text{mm}^2}{\text{s}}$$

These values could be realistic, since in ISO 62 it is said that the diffusion coefficient values of the plastics usually are $10^{-6}\,\frac{\text{mm}^2}{\text{s}}$ at 23°C.

6.2 Effects of the dehumidifying gas on the sample masses

Two different dehumidifying gas were compared: dry nitrogen gas and CDA, in a small scale experiment. Measured masses of the samples are shown in Table 9, during the 10 days experiment.

Table 9. *Measured masses of the samples during the 10 day test series.*

		Mass (mg)							
Tube	**Sample\days**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**10**
N_2	1	57.91	57.84	57.81	57.79	57.77	57.73	57.82	57.83
	2	58.92	58.78	58.8	58.84	58.82	58.79	58.8	58.84
	3	58.99	58.82	58.89	58.87	58.87	58.81	58.89	58.86
	4	57.87	57.71	57.76	57.75	57.79	57.8	57.81	57.8
	5	58.66	58.47	58.5	58.55	58.51	58.52	58.61	58.59
	6	59.13	59.03	59.08	59.02	59.02	59.08	59.08	59.08
CDA	1	58.83	58.71	58.78	58.75	58.72	58.69	58.74	58.72
	2	58.33	58.23	58.28	58.21	58.24	58.24	58.29	58.28
	3	58.41	58.31	58.46	58.37	58.36	58.39	58.43	58.46
	4	58.43	58.27	58.36	58.36	58.33	58.32	58.39	58.39
	5	58.63	58.5	58.58	58.53	58.5	58.5	58.54	58.59
	6	59.57	59.38	59.51	59.49	59.5	59.49	59.53	59.52

The average masses for both sample group during the test are presented in Figure 24.

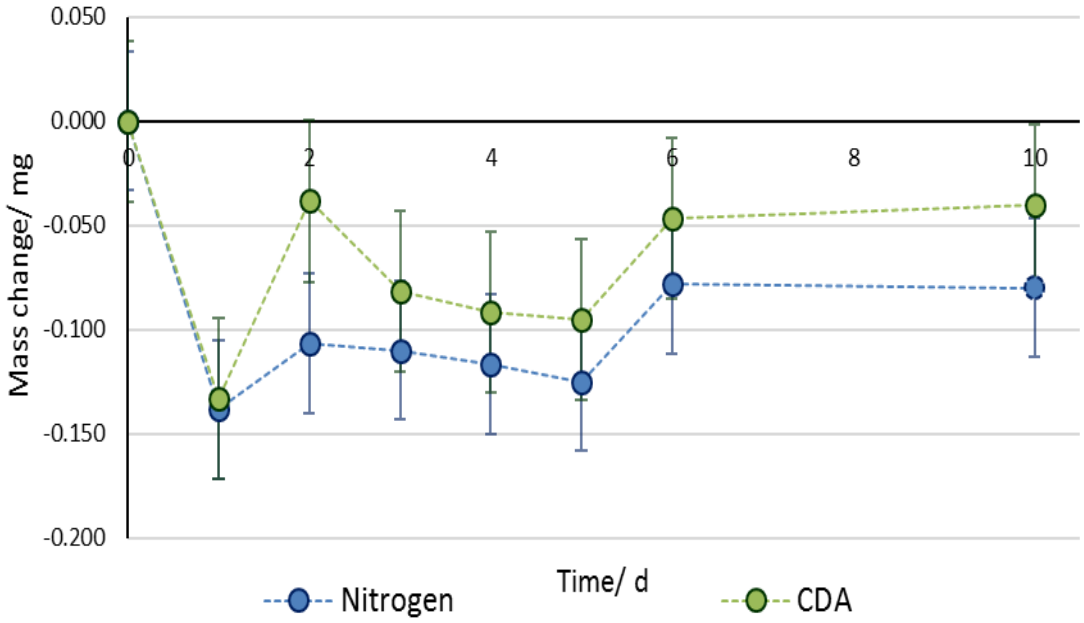

Figure 24. *The average mass changes in the CDA and nitrogen atmosphere in relation to time.*

The humidities of the containers were monitored every three hours and their ability to recover after opening the tubes were studied. The RH in the CDA tube was remained under 4 %, whereas RH of the nitrogen tube was remained under 6.5 %. There were noticed also difference between the minimum values of the RH in the tubes. CDA contained tube the minimum RH value was 1.67, during this test series. When the minimum RH value of the nitrogen contained tube was 3.76. These values are still estimations since the RH was reported in every 3 hours.

In the end of the test, wet and dry mass (after the drying process) were measured from both tubes, and from those values the moisture contents of the samples could be calculated by Formula 6. These results are shown in Table 10.

Table 10. *Calculated moisture contents of the samples inside the two test tubes after 10 days.*

Tube	Sample	Wet mass (mg)	Dry mass (mg)	Mass difference (mg)	Moisture content (%)
N_2	1	57.830	57.860	-0.030	-0.052
	2	58.840	58.860	-0.020	-0.034
	3	58.860	58.920	-0.060	-0.102
	4	57.800	57.810	-0.010	-0.017
	5	58.590	58.570	0.020	0.034
	6	59.080	59.130	-0.050	-0.085
Average		**58.500**	**58.525**	**-0.025**	**-0.043**
CDA	1	58.720	58.740	-0.020	-0.034
	2	58.280	58.270	0.010	0.017
	3	58.460	58.490	-0.030	-0.051
	4	58.390	58.340	0.050	0.086
	5	58.590	58.580	0.010	0.017
	6	59.520	59.520	0.000	0.000
Average		**58.660**	**58.657**	**0.003**	**0.006**

These mass differences were affected a lot of the static electricity of the samples. This was noticed during the measurements in the scale. Static electricity affects in the stabilization of the scale. This static electricity may be a results from clothes of the weigher made from synthetic fiber, disposable gloves or any kind of friction especially in low humidity atmosphere. In addition the error of the scale was relatively big compared to the sample size, why the reading of the scale may have not be fully accurate and may explain partially the negative values of the mass differences and the moisture contents. However, it can be said that the samples were practically dry already before the vacuum drying.

6.3 The recovery time for the gas

The rate for the dehumidification for nitrogen gas and CDA were investigated. The recovery times for CDA and nitrogen were shown in Figure 25.

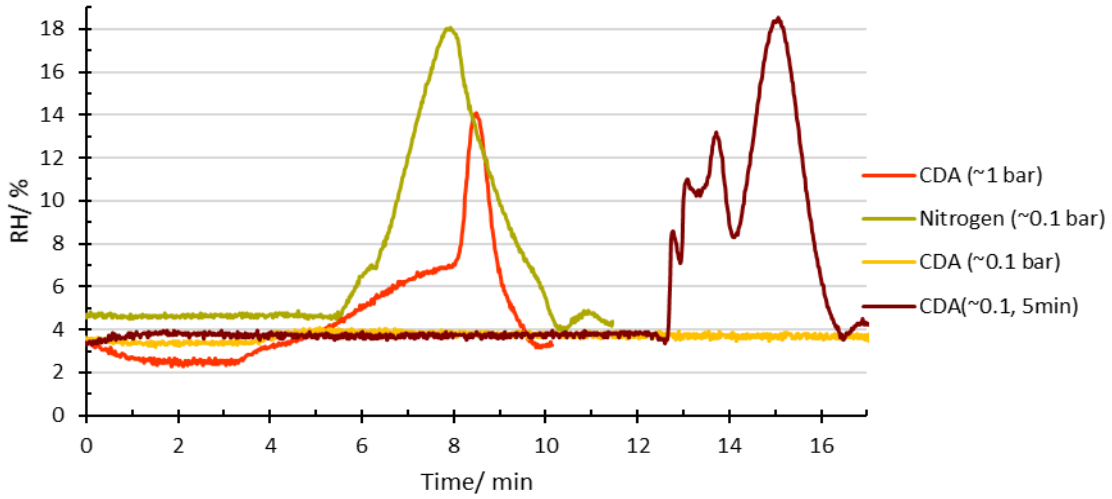

Figure 25. *The recovery times of the moisture for CDA with two pressures and nitrogen after opening the tube.*

Due of these results, the CDA tube with 0.1 bar was kept open also for 5 minutes to see if there would be change. This was long enough period to see change in the RH values after nearly 13 minutes.

7 DISCUSSION

7.1 Absorption rate of PLGA in standard conditions

From the data shown in 6.1, there could notice that there were surprisingly big changes in the absorbed moisture values (in m-%). From the results can be seen that the process phase 2 samples absorbed much more moisture in m-% (2.18 ± 0.67 m-% in 100 % RH and 0.74 ± 0.31 m-% in 50 % RH) than the process phase 1 ones (0.51 ± 0.05 m-% in 100 % RH and 0.17 ± 0.08 m-% in 50 % RH). This is a consequence of the rate between surface area and the volume of the billet. Since process phase 2 samples have more surface area compared to volume, they can absorb more moisture. However, the error of the balance (0.1 mg) should also be considered when reading the results. Due to this, maximum error is under 0.2 m-% units for the absorption rate values.

Even this small amounts of moisture would lead to degradation of the material especially in higher temperatures than the T_g. The preferred maximium amount of moisture before the process phase 1 is 0.02 %, and for the packaged product 0.5 %, or preferably even 0.05 %, as explained in 2.2.3.

The difference between moisture absorption in process phase 1 and the process phase 2 samples is also related to the microscopial surface structure of the billets. Since the surfaces of the process phase 2 samples are uneven at the surface (see Figure 26), the area is even more in relation to the volume and hence increase the absorption rate further.

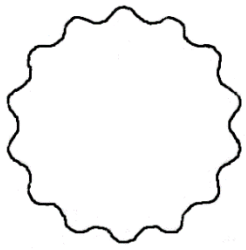

Figure 26. *Picture of the cross section of the process phase 2 sample.*

The microscopial surface structure of the billets also explains why process phase 1 samples have so small deviation. Since the surfaces of the process phase 1 samples are relatively smooth (see Figure 27), the samples are quite homogenous with each other and the moisture absorption is quite identical, as can be seen from the Figure 23.

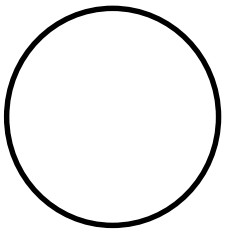

Figure 27. *Picture of the the cross section of the process phase 1 sample.*

Whereas, the surface of the process phase 2 samples are uneven and unidentical. Their unique structure affect in the moisture absorption and lead to wider deviation that can be seen from the Figure 23.

Bigger moisture absorption rate of the process phase 2 samples may also be a result of their orientated nature. During orientation the polymer chains of the material are re-organized as parallel chains, when the moisture may enter the material relatively easily between the polymer chains.

These standard conditions differ from the average laboratory conditions partly. According to FDA, laboratory conditions should be 20 to 25 °C with relative humidity of 35 to 50 % depending on geographical area, to provide comfortable working environment [77]. So the temperature might be slightly over the standard temperature, but still remarkable below the T_g of the considered material, PLGA, hence the difference should not affect considerably. The humidity however, might be even 15 percent units below the standard conditions, which could have significant effect on moisture absorption rate.

7.2 Effects of the dehumidifying gas on the sample masses

Since the nitrogen is relatively expensive, alternate solution was searched and one potential option was found according the literature: CDA. CDA was compared to the nitrogen gas as dehumidifying gas.

As can been seen from the Figure 24, according to this test, there is nearly at all difference in the changes of the masses, between these two type of dehumidifying gas. However, since the mass changes are measured during one week, this experiment is only directional test, that gives the idea that the dry compressed air may be potential option for the nitrogen gas. The differences in the masses after the day three are relatively small changes. The variation in the masses may also be a result of the static electricity in the samples. The static electricity was noticed in the samples after 3 days and after that it was noticed to grow a day after day. The cause of the static electricity might have been very dry atmospheres inside container or/and small friction between the samples and the test tube. Because of the static electricity, the value of the scale stabilized very

slowly, in the worst case, not at all. This made the reading more difficult and could cause error to the measured values.

The standard deviation for both sample groups was nearly the same. Samples that were in CDA atmosphere had only slightly bigger deviation (average standard deviation between the samples was 0,038680) when compared to samples that were in nitrogen atmosphere (average standard deviation between the samples was 0,033290). In this test serie, the difference between the standard deviations is probably result from static electricity and does not relate to the actual gas properties.

When the humidity inside the container were monitored, was noticed that the RH in CDA contained tube was slightly lower than the RH in the nitrogen contained tube during this test series. Both maximum (CDA with 3.93 and nitrogen with 6.23) and minimum (CDA with 1.67 and nitrogen with 3.76) values of the RH of the tubes had significant difference. This may mean that probably the moisture contents of the samples were also lower in the CDA contained tube. However, this could not be confirmed, since the scale was not accurate enough, so that the moisture content inside the samples could have been calculated/measured in a realistic, as can be seen from the Table 10.

When the moisture content of the samples (see Table 10) were calculated by Formula 6, the difference between the samples, that were in CDA, and the samples, that were in nitrogen, was negligible, due to the scale error. According to these result could be said that all of the samples were practically dry. According to the Figure 24, can be said that CDA worked as assumed in the theory (see 2.3.3). Because of the CDA flow, the moisture can get out of the container, that enables effective transportation of moisture, since oxygen of the CDA reacts with water molecules relatively easy. In that way, CDA could be even more effective than the dry nitrogen gas. However, to confirm this accusation, further tests need to be done, with bigger samples and longer test period.

7.3 The recovery time for the gas

Also the capability to dry the container contents were evaluated and the drying rates were measured. As can be seen in Figure 25, CDA with 1 bar dryes the content of the tube faster than nitrogen. The main reason for this was the pressure difference between nitrogen and CDA. Since CDA had 1 bar over pressure, the gas would not easily mixed up in the tube when the tube was opened. The CDA inside the tube was pushed away from the tube, why there was not going so much moist ambient air in, which caused smaller humidity inside the tube and slower rise to the 7 %, when the switch turned open and the moist gas inside the tube is pushed away by the pressure inside the tube. However, when evaluating rate of the drying procedure, can be seen that the slope of CDA curve was way more bigger, so the content inside the tube was drying faster than the nitrogen tube.

In addition to these two measurements, also CDA with ~0.1 bar pressure was measured. However, nearly any change in the RH value was seen. This may be due to that dry air can not hold as much gaseous water molecules compared to nitrogen, so the gas inside the tube will not mixed up as easily. According to another theory, there might have been some moisture in the middle of the tube where there were not any measuring device, and that time just was not enough for it to move and mix with the gas in the end of the tube. Due of these results, the tube kept open for 5 minutes to see if there would be change. During this test the RH was risen nearly to the same scale than in the nitrogen tube with 0.1 bar of pressure. However, it took a longer period of time for the moisture to diffuse to the other end of the tube (near the moisture logger). In addition can be noticed that the RH is decreasing with a more rapid rate, which supports the theory of the CDA's more efficient removal of the water molecules.

7.4 Future studies

During this six months clarity to the moisture absorption has been achieved. However, during these tests some questions rose, why some continued studies should be done.

According to the studies done in 6.3, CDA flow may enable effective transportation of moisture, due to reaction between oxygen and water molecules. Hence, CDA could be even more effective than the dry nitrogen gas. However, to confirm this accusation, further tests need to be done. For example, by repeating the test with bigger samples (more than 1 g) and longer test period (at least two weeks). In addition, more accurate scale could be used.

In addition, nitrogen would protect the chemical structure of the molecule more carefully, since nitrogen is really inert molecule, so it does not want to react with the polymer chains in the material. Whereas, air contains approximately 21 % of oxygen that is relatively reactive molecule compared to nitrogen, so it would more easily react with the polymer chain and cut into oligomer chains. However, this type of reactions are not very frequent.

8 CONCLUSION

PLGA is one of the most studied and used biodegradable polymer in orthopedic field. The aim of this study was to examine the water absorption rate of PLGA (according to ISO62:2008) for process phase 1 and process phase 2 samples. In addition, comparison between two different dehumidifying gases for the production was made.

During the processing phase the material should be as dry as possible to prevent hydrolysis. According the moisture absorption rate tests the process phase 2 samples absorbed moisture 0.51 ± 0.05 m-% and the process phase 1 samples 2.18 ± 0.67 m-% at 100 % RH and 23 ℃, during 24 hours. Whereas, the process phase 2 samples absorbed moisture 0.74 ± 0.31 m-% and the process phase 1 samples 0.17 ± 0.08 m-% at 50 % RH and 23 ℃, during 24 hours. These amounts of moisture seem to be relatively small, and they could be used as products straight away (moisture content < 0.5 m-%). However, these samples could not be used for high temperature processing without pre-drying the samples. Reasons for these differences may be e.g. the orientated nature of process phase 2 samples and the bigger surface area in relation to volume in the process phase 2 samples.

According to the directional tests that had done, compressed dry air could be potential option for the nitrogen gas in different subprocesses, since it is capability to dry and preserve samples was relatively similar to nitrogen. In addition, some advantages were noticed compared to nitrogen. Such as, more efficient water removal capability of the oxygen molecules, due to reactivity of the oxygen molecules with the water molecules.

During this six months clarity to the moisture absorption has been achieved. However, during these tests some questions was risen, and hence some continued studies should be done.

9 BIBLIOGRAPHY

[1] J. Davis, "Overview of Biomaterials and Their Use in Medical Devices," in *HANDBOOK OF MATERIALS FOR MEDICAL DEVICES,* ASM International, 2003.

[2] J. Park and S. Kang, "A study on surface, thermal and mechanical properties of absorbable PLGA plate," *International Journal of Control and Automation,* vol. 6, no. 6, pp. 73-82, 2013.

[3] T. Wang, M. Jiang and Y. Wu, "Nanoparticles composed of PLGA and hyperbranched poly (Amine-ester) as a drug carrier," *Journal of Polymer Research,* vol. 17, no. 3, pp. 335-345, 2010.

[4] R. Dorati, C. Colonna, I. Genta, T. Modena and B. Conti, "Investigation of the degradation behaviour of poly(ethylene glycol-co-d,l-lactide) copolymer," *Polymer degradation and stability,* vol. 92, no. 9, pp. 1660-1668, 2007.

[5] Ensinger-online, "Moisture absorption," Ensinger-online, [Online]. Available: http://www.ensinger-online.com/nl/technical-information/properties-of-plastics/various-other-properties/moisture-absorption/. [Accessed 11 2015].

[6] Food and drug administration, "What is a medical device?," FDA, 7 7 2015. [Online]. Available: http://www.fda.gov/AboutFDA/Transparency/Basics/ucm211822.htm. [Accessed 11 2015].

[7] A. Kumar, K. Karthick and K. Arumugam, "Biodegradable Polymers and Its Applications," *International Journal of Bioscience, Biochemistry and Bioinformatics,* vol. 1, no. 3, 2011.

[8] H. Makadia and S. Siegel, "Poly Lactic-co-Glycolic Acid (PLGA) as Controlled Drug Delivery Carrier," *Polymers,* vol. 3, pp. 1377-1397, 2011.

[9] Euroopan yhteisö, "NEUVOSTON DIREKTIIVI," 20 7 1990. [Online]. Available: http://eur-lex.europa.eu/legal-content/FI/TXT/PDF/?uri=CELEX:31990L0385&from=FI. [Accessed 11 2015].

[10] EU Law and Publications, "NEUVOSTON DIREKTIIVI 93/42/ETY," 11 10 2007. [Online]. Available: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1993L0042:20071011:fi:PDF. [Accessed 11 2015].

[11] Euroopan yhteisö, "EUROOPAN PARLAMENTIN JA NEUVOSTON DIREKTIIVI 98/79/EY," 7 12 1998. [Online]. Available: http://eur-lex.europa.eu/legal-content/FI/TXT/PDF/?uri=CELEX:31998L0079&from=FI. [Accessed 11 2015].

[12] Euroopan unioni, "EUROOPAN PARLAMENTIN JA NEUVOSTON DIREKTIIVI 2007/47/EY,," 21 9 2007. [Online]. Available: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:247:0021:0055:FI:PDF. [Accessed 11 2015].

[13] J. Middleton and A. Tipton, "Synthetic biodegradable polymers as orthopaedic devices," *Biomaterials,* vol. 21, pp. 2335-2346, 2000.

[14] L. McKeen, "Plastics used in medical device," in *Handbook of polymer applications in medicine and medical devices*, Elsevier Inc, 2014, pp. 21-53.

[15] A. a. S. M. Södergård, "Properties of lactic acid based polymers and their correlation with composition," *Progress in Polymer Science,* vol. 27, pp. 1123-1163, 2002.

[16] L. Xiao, B. Wang, G. Yang and M. Gauthier, "Poly(Lactic Acid)-Based Biomaterials: Synthesis, Modification and Applications," in *Biomedical Science, Engineering and Technology*, In Tech, 2012, p. 250.

[17] M. Broz, D. VanderHart and N. Washburn, "Structure and mechanical properties of poly(d,l-lactic acid)/poly(ε-caprolactone) blends," *Biomaterials,* vol. 24, no. 23, p. 4181–4190, 2003.

[18] J. Park and S. Im, "Phase behavior and morphology in blends of poly(L-lactic acid) and poly(butylene succinate)," *Journal of applied polymer science,* vol. 86, no. 3, pp. 647-655, 2002.

[19] J. Anderson and M. Shive, "Biodegradation and biocompatibility of PLA and PLGA microspheres," *Advanced Drug Reviews,* vol. 28, no. 1, pp. 5-24, 1997.

[20] W. Jiang, R. Gupta, M. Deshpande and S. Schwendeman, "Biodegradable poly(lactic-co-glycolic acid) microparticles for injectable delivery of vaccine antigens," *Advanced Drug Delivery Reviews*, vol. 57, no. 3, pp. 391-410, 2005.

[21] S. Vainionpä, P. Rokkanen and P. Törmälä, "Surgical applications of biodegradable polymers in human tissues," *Progres in Polymer Science*, vol. 14, pp. 679-716, 1989.

[22] S. Shalaby and R. Johnsson, Synthetic absorbable polyesters, New York: Hanser, 1994.

[23] NPCS Board of Consultants & Engineers, "Medical device packaging," in *Handbook on Medical and Surgical Disposable Products*, Delhi, Niir Project Consultancy Services, 2014, p. 87.

[24] G. Akovali, "Plastics use in healthcare and their possible health effects," in *Plastics, Rubber and Health*, Shropshire, iSmithers Rapra Publishing, 2007, pp. 131-132.

[25] Styrolution, "Products," [Online]. Available: https://www.styrolution.com/Products.html. [Accessed 11 2015].

[26] Sigma-Aldrich, "Acrylic acid," Sigma-Aldrich, 2015. [Online]. Available: http://www.sigmaaldrich.com/catalog/product/aldrich/147230?lang=fi&region=FI. [Accessed 4 2016].

[27] Evonik industries, "Acrylic Advisor - Technical reference guide for ACRYLITE® & ACRYMID® acrylic polymers," 2014. [Online]. Available: http://www.acrylite-polymers.com/product/acrylite-polymers/Documents/evonik-acrylic-advisor.pdf. [Accessed 30 April 2016].

[28] H. Deng, C. Reynolds, N. Cabrera, N. Barkoula, B. Alcock and T. Peijs, "The water absorption behaviour of all-polypropylene composites and its effect," *Composites: Part B*, p. 268–275, 11 March 2010.

[29] BASF Corporation , "Tensile Properties of Semi-Crystalline Thermoplastics –," BASF Corporation, New Jersey, 2003.

[30] Sigma-Aldrich, "Polypropylene," [Online]. Available: http://www.sigmaaldrich.com/catalog/product/aldrich/428116?lang=fi&region=FI. [Accessed 12 2 2016].

[31] Matweb, "Borealis Bormed Polypropylene copolymer for medical applications," [Online]. Available: http://www.matweb.com/search/datasheettext.aspx?matguid=dfa75b9c548b4fe6993daf16c5314aef. [Accessed 2016 2 12].

[32] D. Carlsson and D. Wiles, "The Photooxidative Degradation of Polypropylene. Part I. Photooxidation and Photoinitiation Processes," *Journal of Macromolecular Science, Part C: Polymer Reviews,* vol. 14, no. 1, pp. 65-106, 1976.

[33] D. Feldman, "Polymer weathering: Photo-oxidation," *Journal of polymers and the environment,* vol. 10, no. 4, pp. 163-173, 2002.

[34] Mettler Toledo, "Moisture guide for plastics, An overview of methods," Mettler Toledo, Greifensee, Switzerland, 2014.

[35] W. Tsai and H. Hang, "Hygrothermal behaviour," in *Introduction to composite materials,* USA, Technomic, 1980.

[36] Plastics technology, "Latest Energy-Saving Technologies," Plastics technology, 2015. [Online]. Available: http://www.ptonline.com/knowledgecenter/Plastics-Drying/Drying-Basics/Energy-Saving-Technologies. [Accessed 11 2015].

[37] Plastic technology, "Resin Dryers: Which Type Is Right for You?," Plastic technology, 3 2005. [Online]. Available: http://www.ptonline.com/articles/resin-dryers-which-type-is-right-for-you. [Accessed 11 2015].

[38] J. Crank, "The mathematics of diffusion," Oxford University press, 1975.

[39] Q. Cai, G. Shi, J. Bei and S. Wang, "Enzymatic degradation behavior and mechanism of poly(lactide-co-glycolide) foams by trypsin.," *Biomaterials,* vol. 24, no. 4, pp. 629-638, 2003.

[40] Middleton, J., "Tailoring of Poly(lactide-co-glycolide) to Control Their Properties for Medical Device and Pharmaceutical Applications," [Online]. Available: http://www.fda.gov/downloads/MedicalDevices/NewsEvents/WorkshopsConferences/UCM335606.pdf. [Accessed 11 2015].

[41] WHAT-WHEN-HOW In Depth Tutorials and Information, "Nano-indentation Studies of Polyglactin 910 Monofilament Sutures (MEMS and Nanotechnology)," [Online]. Available: http://what-when-how.com/mems-and-nanotechnology/nano-indentation-studies-of-polyglactin-910-monofilament-sutures-mems-and-nanotechnology/. [Accessed 8 4 2016].

[42] British Plastics Federation, "Drying of Polymer," British Plastics Federation, 2015. [Online]. Available: http://www.bpf.co.uk/plastipedia/processes/Drying_of_Polymer.aspx. [Accessed 11 2015].

[43] Plastics technology, "Drying," Plastics technology, 2015. [Online]. Available: http://www.ptonline.com/knowledgecenter/Clear-Engineering-Polymers/Processing-polymers/Drying-polymers. [Accessed 11 2015].

[44] C. Macdermott and A. Shenoy, Selecting Thermoplastics for Engineering Applications, New York: Marcel Dekker Inc., 1997.

[45] RTP Company, "Molding and Processing Guidelines for PLA (Polylactic Acid) Compounds," RTP Company, [Online]. Available: http://www.rtpcompany.com/technical-info/molding-guidelines/pla-compounds/. [Accessed 8 March 2016].

[46] Poly-med Inc., "Absorbable Polymer Processing: Keys from the Field," 16 9 2013. [Online]. Available: http://poly-med.com/absorbable-polymer-processing-keys-field/. [Accessed 11 2015].

[47] N. Passerini and D. Craig, "An investigation into the effects of residual water on the glass transition temperature of polylactide microspheres using modulated temperature," *Journal of control release,* vol. 73, pp. 111-115, 2001.

[48] T. Hanafy, "Advances in Materials Physics and Chemistry," *Miscibility Behavior of Poly Ethylene Glycol or Poly Ethylene Succinate/Chlorinated Poly Vinyl Chloride Blends Studied by Dielectric Relaxation Spectroscopy,* vol. 3, no. 2, pp. 97-104, 2013.

[49] Terrauniversal, "Desiccator Storage: The Missing Link in the Manufacturing Chain," 4 1999. [Online]. Available: https://www.terrauniversal.com/uploads/tech_resources/1999_desiccator_white_paper_081415130640.pdf. [Accessed 11 2015].

[50] A. Felgner, "Basics of Karl Fischer Titration," 2012. [Online]. Available: https://www.sigmaaldrich.com/content/dam/sigma-aldrich/countries/czech/2_basics.pdf. [Accessed 11 2015].

[51] SI-analytics, "Karl-Fischer Titration – the method for determining water," [Online]. Available: http://www.si-

analytics.com/fileadmin/upload/Informationen/Titration/INT/Technical-Data_TitroLine-KF-trace_742-KB_PDF-English.pdf. [Accessed 11 2015].

[52] Mettler Toledo, "Brochure: Guide to moisture analysis," Mettler Toledo, [Online]. Available: http://us.mt.com/us/en/home/supportive_content/product_documentation/product_brochures/po/labtec/05_Moisture_Analyzer/00_Family/01_Brochures/Guide_to_Moisture_Analysis_Request.html. [Accessed 11 2015].

[53] Mettler Toledo, "Measuring Principle," Mettler Toledo, [Online]. Available: http://us.mt.com/us/en/home/microsites/moisture-check/Guide_analyse/meas_principal.html#panel-1. [Accessed 11 2015].

[54] Ceramicx, "Fundamentals of IR," Ceramicx, 2015. [Online]. Available: http://www.ceramicx.com/infrared-heat/. [Accessed 11 2015].

[55] Plastics technology, "OVERVIEW:Drying," Plastics technology, [Online]. Available: http://www.ptonline.com/zones/drying#TB_inline?height=400&width=500&inlineId=FullZoneInfo. [Accessed 11 2015].

[56] A. Hailwood and S. Hoorbin, "Absorption of water by polymers: analysis in terms of a simple model," *Transactions of the Faraday Society*, vol. 42, pp. B084-B092, 1946.

[57] A. Martell, "Entropy and the second law of thermodynamics," *Journal of Chemical Education*, vol. 23, no. 4, p. 166, 1946.

[58] Simar, "The Drying of Engineering Plastics," [Online]. Available: http://www.simar-int.com/files/vortrag_engl.pdf. [Accessed 11 2015].

[59] SAE International, "Driving out moisture via nitrogen purging," SAE International, [Online]. Available: http://articles.sae.org/9130/. [Accessed 11 2015].

[60] John's corner technical blog, "Drying – Basic Hot Air Dryer Design," Cleaning Technologies Group, 2014. [Online]. Available: http://www.ctgclean.com/tech-blog/2013/08/drying-basic-hot-air-dryer-design/. [Accessed 8 February 2016].

[61] Plastics technology, "How to Dry PET for Container Applications," Plastics technology, [Online]. Available: http://www.ptonline.com/articles/how-to-dry-pet-for-container-applications. [Accessed 11 2015].

[62] Plastics technology, "Compressed Air Dryers," Plastics technology, [Online]. Available: http://www.ptonline.com/knowledgecenter/Plastics-Drying/Dryer-Types/Compressed-Air-Dryers. [Accessed 11 2015].

[63] Plastics technology, "Membrane Dryers," Plastics technology, 2015. [Online]. Available: http:/www.ptonline.com/knowledgecenter/Plastics-Drying/Dryer-Types/Membrane-Dryers. [Accessed 11 2015].

[64] WALMEC North America, "How A Membrane Dryer Works," WALMEC North America, 2016. [Online]. Available: http://www.walmecna.com/how-membrane-works.php. [Accessed 15 5 2016].

[65] A. Mujumdar, "Drying at high temperature: the effect of internal pressure on mass transfer," in *Handbook of Industrial Drying, Fourth Edition*, Boca Raton, CRC Press, 2014, p. 815.

[66] Shaffer, R., "How Dryers Work," Conair group, [Online]. Available: http://www.conairgroup.com/assets/Knowledge-Center/Whitepapers/Drying/Conair-HowDryersWork.pdf. [Accessed 11 2015].

[67] Plastics technology, "Vacuum dryers," Plastics technology, 2015. [Online]. Available: http://www.ptonline.com/knowledgecenter/Plastics-Drying/Dryer-Types/Vacuum-Dryers/. [Accessed 11 2015].

[68] LABORATORY OF CATALYSIS AND ORGANIC SYNTHESIS, "Working correctly with the nitrogen line," [Online]. Available: http://lcso.epfl.ch/files/content/sites/lcso/files/load/Nitrogen%20Line.pdf. [Accessed 11 2015].

[69] Pubchem, "Nitrogen," National Center for Biotechnology Information, 16 September 2004. [Online]. Available: https://pubchem.ncbi.nlm.nih.gov/compound/Nitrogen#section=Top. [Accessed 15 May 2016].

[70] a.hak industrial service, "Drying," [Online]. Available: http://www.a-hak-is.com/en/home/what_we_do/services/pipeline_services/drying. [Accessed 11 2015].

[71] Freudenberg-NOK, "Efficiently Dry Compressed Air," Freudenberg-NOK, 19 2 2014. [Online]. Available: http://hydraulicspneumatics.com/other-technologies/efficiently-dry-compressed-air. [Accessed 11 2015].

[72] K. Wakamatsu, Y. Shimizu, T. Kato and I. Takahashi, "Air purifier". United States of America Patent US 3804942 A, 16 April 1974.

[73] Parker, "Sample Concentration by Evaporation of the Solvent with Nitrogen," 11 2012. [Online]. Available: http://www.parker.com/literature/Balston%20Filter/AGS/AGS%20Case%20Studies/PDFs/Nitrogen_for_Sample_Concentration.pdf. [Accessed 11 2015].

[74] "Nitrogen Enhanced Purging Systems (NEPS) Technical Information," AGM Container Controls, 2016. [Online]. Available: http://www.agmcontainer.com/neps-technical-information. [Accessed 15 May 2016].

[75] "Polymer Degradation," [Online]. Available: http://sunil.cusat.ac.in/pages/pdf/Polymer%20Degradation.pdf. [Accessed 30 April 2016].

[76] Kemianteollisuus ry, *Plastics- Determination of water absorption (ISO 62:2008),* Brussels: Suomen standardisoimisliitto SFS, 2008.

[77] U.S. Food and Drug Administration, "Facilities and Environmental Conditions (linked to Vol. III, sec. 2) ORA-LAB.5.3," 10 1 2003. [Online]. Available: http://www.fda.gov/ScienceResearch/FieldScience/ucm171875.htm. [Accessed 25 4 2016].

ATTACHMENT A: TEST REPORT ACCORDING TO SFS-EN ISO 62:2008 (19.-22.01.2016)

Material

As a material, PLGA was used.

Samples

Samples were made and cut beforehand (means of measuring the dimensions of the test samples to an accuracy of ± 0.1 mm) and stored in container in nitrogen atmosphere.

Methods

Methods 1 and 4 were used.

Method 1: From nitrogen atmosphere, samples were transported in sealed aluminum foil bag and placed into Petri dish, that is placed in the vacuum oven (WBT Binder VD115, vacuum pump: Trivac D 4 B) in a room temperature (vacuum can be sucked as low as 0×100mbar) for 24 ± 0.17 h. After the vacuum drying, the samples were put into desiccator (VWR Prolabo, Chameleon® Silica gel 2-6mm) during the transportation, and weighted by balance (TEOPAL, Precisa 240A), which has an accuracy of ± 0.1 mg. After the weighting, samples were moved into environmental chamber (OZONE GENTLE, Model: PR-1KPH) in individual stainless steel cages in 5 liters beaker. In the cabinet the atmosphere conditions are following: In test number one, temperature 23℃ and relative humidity is 100 % (6 samples × 300 ml of distilled water). After 24 hours, samples were weighted during under 1 minute. From the weighted masses, absorbed moisture contents were calculated.

Method 4: Preparations were exatly same as in method 1 until the first weighing. After the first weighting, samples were moved into environmental chamber (OZONE GENTLE, Model: PR-1KPH) in individual Petri dishes. In the cabinet the atmosphere conditions were following: temperature 23℃ and relative humidity is 50 %. After 24 hours, samples were weighted during under 1 minute. From the weighted masses, absorbed moisture contents were calculated.

Water absorption

Moisture content was calculated by the following formula:

$$Absorbed\ moisture\ content\ (\%) = c \times 100\% = \frac{m_{wet} - m_{dry}}{m_{dry}} \times 100\% \quad (1)$$

Absorbed moisture contents is presented in Table 11 and Table 12.

Table 11. *Absorbed moisture contents in 23℃ and in 100% RH.*

Sample	Absorbed moisture (m-%)
1	2,700096432
2	1,434034417
3	2,396931927
4	0,464037123
5	0,506146059
6	0,558659218

Table 12. *Absorbed moisture contents in 23℃ and in 50% RH.*

Sample	Absorbed moisture (m-%)
1	0,967117988
2	0,87124879
3	0,385356455
4	0,120627262
5	0,116795141
6	0,259006357

Water absorption at saturation

From Fick's law there is a following connection between moisture content c and moisture content at saturation c_s:

$$\frac{c}{c_s} \leq 0.51 \tag{2}$$

Moisture content at saturation c_s is calculated with the formula 2 for both process phase 1 and process phase 2 samples.

Process phase 1 sample:

$$c \leq 0.51 \times c_s$$

$$c_s \geq \frac{c}{0.51}$$

$$c_s \geq \frac{0.00165476254}{0.51}$$

$$c_s \geq 0.0032446 \ldots$$

Process phase 2 sample:

$$c_s \geq \frac{0.00741241078}{0.51}$$

$$c_s \geq 0.0145341 \ldots$$

Diffusion coefficient

This formula can be said also in different form:

$$\frac{D\pi^2 t}{d^2} \leq 0.50 \tag{3}$$

where *t* is the duration of immersion of the test sample in the water or humid air, in seconds, D is the diffusion coefficient and *d* is the thickness of the test sample, in millimeters.

Diffusion coefficient is calculated with the formula 3 for both process phase 1 and process phase 2 samples.

Process phase 1 sample:

$$\frac{D\pi^2 t}{d^2} \leq 0.50$$

$$D\pi^2 t \leq 0.50 \times d^2$$

$$D \leq \frac{0.50 \times d^2}{\pi^2 t}$$

$$D \leq \frac{0.50 \times 4{,}13\text{mm}^2}{\pi^2 \times 86477{,}5\text{s}} = 9{,}9923 \ldots \times 10^{-6} \frac{\text{mm}^2}{\text{s}}$$

Process phase 2 sample:

$$D \leq \frac{0.50 \times 2{,}09\text{mm}^2}{\pi^2 \times 86477{,}5\text{s}} = 2{,}5589 \ldots \times 10^{-6} \frac{\text{mm}^2}{\text{s}}$$

These values could be realistic, since in ISO 62, there have informed that the diffusion coefficient values of the plastics usually are $10^{-6} \frac{\text{mm}^2}{\text{s}}$ at 23°C

Incidents

Everything went as planned, without any incidents.